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PATHOGENESIS OF INTESTINAL AMEBIASIS IN KITTENS*

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The principal object of the observations and experiments recorded in this paper was to learn the nature of the attack of *Endameba histolytica* on the colon in kittens. Since, therefore, the emphasis is placed on the origin and the development of the lesions, the term "pathogenesis" is used rather than "pathology."[1] Pathogenesis also concerns the effects on the parasite of the reactions of the host. Evidences of this reaction may be studied in the endamebas because their nuclei, like those of the body cells, pass through various stages of necrosis after death. The plan of procedure was to inoculate kittens in several well tested ways, to kill them at intervals of from eighteen hours to thirty days afterward, to fix the tissues of the colon and to section and stain them. The limitations of this method, particularly in the field of pathogenesis, were clearly recognized. One had to draw conclusions from the appearance of fixed material as to the reaction that had occurred during life. As the work progressed, it was found practicable to study the lesions in the living colon for a short time after its removal from the body. It is hoped that other ways of using living material may soon be devised.

The problem was suggested by Dr. Robert Hegner. Laparotomies were performed on the kittens, as one method of infecting them. A technic developed and demonstrated by Dr. Justin Andrews was used. It involved the use of sterilized instruments, towels, gauze, tape, and other such paraphernalia, and aseptic precautions. The kittens recovered quickly, and there was seldom any peritonitis.

REVIEW OF THE LITERATURE

The susceptibility of the kitten to amebiasis following rectal injection of material from human dysenteric stools was demonstrated by Hlava.[2]

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1. "Pathogenesis has reference to the generation and development of disease."—Encyclopedia Brittanica. "Pathological disturbances are the result of some form of injury or of the immediate or more remote reactions of the body to injury."—MacCallum, 1925.

2. Hlava: Dysenterie in Bohemia (article in Bohemian); abstract in Centralbl. f. Bacteriol **1**:537, 1887.

Later investigations established the following facts: (1) the nonpathogenicity for kittens of the bacterial flora of these dysenteric stools (Kartulis [3]), (2) the noninfectivity, when administered via the mouth, of dysenteric feces containing trophozoites but not cysts (Quincke and Roos [4]), (3) the pathogenicity of endamebas from bacteria-free abscesses of the human liver (Kruse and Pasquelle [5]), (4) the production of amebic abscesses of the liver in kittens (Marchioux [6]), (5) the occurrence in the human bowel of several species of endamebas, some pathogenic in kittens and others not so (Schaudinn,[7] Craig,[8] 1905; Viereck [9]), (6) the maintenance of viability and pathogenicity by *E. histolytica* when passed through a series of kittens (Wenyon,[10] and Dale and Dobell [11]), (7) the invasion of the blood stream of cats infected with amebiasis by bacteria (Sellards and Baetjer [12]), (8) the excystation of the parasite in the colon when the cysts are injected through its wall following a laparotomy in which stasis is produced by a ligature (Sellards and Theiler [13]). It is also agreed that the kitten does not harbor endamebas except when experimentally infected, and that cysts are never produced in the colon of the cat, so that the latter never becomes a carrier. Attempts to determine the occurrence or nonoccurrence of diverse strains of *E. histolytica,* some extremely pathogenic in cats and others not so, have led to conflicting views

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3. Kartulis, S.: Ueber tropische Leberabscesse und ihr Verhältniss zur Dysenterie, Virchows Arch. f. path. Anat. **118**:97, 1889; Ueber weitere Verbreitungsgebiete der Dysenterie Amoeben, Centralbl. f. Bacteriol. **7**:54, 1890; Einiges ueber die Pathogenesse der Dysenterie Amoeben, ibid. **9**:365, 1890.

4. Quincke, H., and Roos, E.: Ueber Amoeben Enteritis, Berl. klin. Wchnschr. **30**:1089, 1893.

5. Kruse, W., and Pasquelle, A.: Untersuchungen ueber Dysenterie und Leberabscess, Ztschr. f. Hyg. u. Infectionskrankh. **10**:161, 1894.

6. Marchioux, E.: Note sur la dysenterie des pays chauds, Compt. rend. Soc. de biol. **51**:870, 1899.

7. Schaudinn, F.: Untersuchungen ueber die Fortpflanzung einiger Rhizopoden, Arb. a. d. Gsndhtsamte. **19**:548, 1903.

8. Craig, C. F.: Entamoeba coli and Entamoeba dysenteriae, Am. Med. **9**:854, 897, 936, 1905.

9. Viereck, H.: Studien ueber die in der Tropen erworbem Dysenterie, Arch. f. Schiffs- u. Tropen-Hyg. **11**:1 (suppl.) 1907.

10. Wenyon, C. M.: Experimental Amoebic Dysentery and Liver Abscess in Cats, J. London Sch. Trop. Med. **2**:27, 1912.

11. Dale, H., and Dobell, C.: Experiments in the Therapeutics of Amebic Dysentery, J. Pharmacol. & Exper. Therap. **10**:399, 1917.

12. Sellards, A. W., and Baetjer, W. A.: The Behavior of Amoebic Dysentery in Lower Animals and Its Bearing upon the Interpretation of Clinical Symptoms of the Disease in Man, Bull. Johns Hopkins Hosp. **25**:238, 1914.

13. Sellards, A. W., and Theiler, M.: Investigations Concerning Amoebic Dysentery, Am. J. Trop. Med. **4**:309, 1924.

(Brumpt,[14] Wagener and Thompson,[15] Kessel [16] and others). Dobell and Laidlaw [17] claim to have developed a noninfective strain in culture by adding rice starch. This was accomplished after thirteen subcultures over a period of thirty-eight days. I [18] was unable to confirm these results.

It is generally agreed that the age during which kittens are most susceptible extends from the time of weaning to the attainment of a weight of from 600 to 700 grams. Investigators report from 50 per cent to 75 per cent of cats positive during this period. Unweaned kittens and older cats are rather refractive. If infection occurs in older cats, it is usually mild and the animal recovers (Sellards and Leiva [19]).

Boeck and Drbohlav [20] conducted a series of experiments which demonstrated that the cultivation of *E. histolytica* in vitro is a relatively simple procedure. This work is of outstanding importance. It relieves the experimenter of dependence on a haphazard supply of the parasite. As pointed out by Wenyon,[21] the aforementioned experiments also enforced a change of viewpoint concerning the pathogenicity of *E. histolytica*. It was formerly thought that the organism could not long endure without access to living tissues. More recent work (Dobell and Laidlaw [17] and Yorke and Adams [22]) has shown that the phenomena of encystment and excystation, which complete the known life cycle, may occur in the test tube.

Studies of the clinical course of amebiasis in kittens and of its pathology have not in any instance been adequately pursued. Controls were never kept, though the kittens were usually purchased in the streets. Such animals are undernourished and usually heavily infected with nematodes, cestodes, coccidia and ectoparasites. Kittens are subject

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14. Brumpt, E.: Etude sommaire de l'*Entamoeba dispar* n. sp. amibe a Kystes quadrinuclees parasite de l'homme, Bull. Acad. de med., Paris **94**:943, 1925.

15. Wagener, E. H., and Thompson, M.: Experimental Amoebiasis in Cats from Acute and Chronic Human Cases, Univ. California Pub. Zool. **26**:267, 1924.

16. Kessel, J. F.:Amoebiasis in Kittens Infected with Amoebae from Acute and "Carrier" Human Cases and with Tetranucleate Amoebae of the Monkey and of the Pig, Am. J. Hyg. **8**:311, 1927.

17. Dobell, C., and Laidlaw, P. P.: On the Cultivation of *Endamoeba histolytica* and Some Other Entozoic Amoebae, Parasitology **18**:283, 1926.

18. Rees, C. W.: The Infectivity and Pathogenicity of a Starch-Fed Strain of *Endamoeba Histolytica*, J. Parasitol. (to be published).

19. Sellards, A. W., and Leiva, L.: Investigations Concerning the Treatment of Amoebic Dysentery, Philippine J. Sc. **22**:1, 1923.

20. Boeck, W. C., and Drbohlav, J.: The Cultivation of *Endamoeba histolytica*, Am. J. Hyg. **5**:371, 1925.

21. Wenyon, C. M.: Protozoology, New York, 1926.

22. Yorke, W., and Adams, A. R. D.: Observations on *Endamoeba histolytica*, Ann. Trop. Med. **20**:279, 1926.

to pneumonia and bacillary dysenteries, particularly during the period when most susceptible to amebic dysentery. And yet it appears from the protocols that careful examinations of the contents of the stomach and the small intestines were not made. The aforementioned parasites were not noted as being present. Small kittens adapt themselves poorly to solitary confinement in wire cages; frequently the controls die, sometimes before the experiments are under way.

The foregoing review of experimental amebiasis in kittens is given because a statement of the present status of the disease is indicated in order to show the bearing on it of the work here set forth. In the field of pathogenesis, as the term is defined in this article, one finds only theories. The first was proposed by Schaudinn,[7] whose name *histolytica* suggests a cytolysin. He held that the penetration of the tissue is made possible for *E. histolytica* because the organism thrusts out, explosively, bladelike pseudopodia consisting only of ectoplasm, which are the weapons of attack. These organella differ from the blunt pseudopodia of *E. coli,* which are thrust out slowly and into which the endoplasm flows. Another theory by Councilman and Lafleur [23] and Wenyon (1912,[10] 1925 [21]) presupposes that the trophozoites make their way to the fundi of the crypts of Lieberkühn of the colon. Here they multiply. The gland responds by excessive secretion of mucus and becomes occluded. A cytolytic toxin secreted by the parasites causes necrosis of the epithelial cells of the gland, and thus an abscess is produced, consisting of a yellowish plug of necrotic material containing endamebas. By pressure and continued secretion, the endamebas may break through the muscularis mucosae into the submucosa, or the plug of necrotic material may be discharged, causing the flecks of bloody mucus which occur in the stools.

Dobell [24] described the earliest lesion as a hyperemic patch of the mucosa. The endamebas working in this area are the cause of this inflammation. They secrete a toxin which dissolves away the cells of the mucosa. In this way pools of cytolysed fluid are produced in which the amebas multiply. It is of interest in this connection that Craig [25] extracted a cytolytic substance in absolute alcohol from masses of *E. histolytica* grown in culture.

The theories are in conflict in essential particulars, though the last two agree in postulating some kind of secretion by the parasite. The

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23. Councilman, W. T., and Lafleur, H. A.: Amoebic dysentery, Johns Hopkins Hosp. Rept. **2**:293, 1891.

24. Dobell, C.: Protozoal Diseases, in Byam and Archibald: Practice of Medicine in the Tropics, London, 1922, vol 2, p. 1335.

25. Craig, C. F.: Observations upon the Hemolytic, Cytolytic and Complement-Binding Properties of Extracts of *Endamoeba histolytica,* Am. J. Trop. Med. **7**:225, 1927.

authors do not supply figures of the progressive stages in these processes or other adequate evidence to substantiate their theories. It seems obvious from this review that further research is indicated to determine to what extent any of the theories coincides with the facts.

MATERIAL AND METHODS

A strain of *E. histolytica* in culture obtained from Captain J. H. St. John of the Army Medical School, Washington, D. C., and established in this laboratory was available. It was cultivated in Boeck and Drbohlav's[20] egg slant serum

TABLE 1.—*Experiments with Laparotomy. (The Endamebas Inoculated Were Cultured in Boeck & Drbohlav's Medium Without Starch.)*

Kitten	Date of Operation	Subculture in Starch	Kitten's Weight, Gm.	Fluid Injected, Cc.	Lapse of Time, Hrs.	Cat died (D.) Cat Killed (K.)	Degree of Infection	Character of Lesions
1*	Oct. 4	....	600	0	44	K.	—	None
2	Oct. 19	2d	600	3	140	D.	++	Mucosa necrotic; diphtheric membrane; nests of amebas in submucosa
3	Oct. 22	3d	600	3	90	K.	+++	Mucosa entirely necrotic; diphtheric membrane; submucosa greatly thickened and invaded
4	Oct. 26	4th	640	3	27	K.	+	Normal
5	Oct. 30	6th	585	3	48	K.	+++	Necrosis of superficial cells of mucosa over entire colon; submucosa thickened
6	Nov. 3	8th	700	3	36	K.	—	Normal; not sectioned
7	Nov. 3	8th	450	1	27	K.	—	Normal; not sectioned
8	Nov. 3	8th	400	2	28	K.	—	Normal; necrosis not demonstrable
9	Nov. 3	8th	340	1	65	K.	—	Lesions of mucosa not observable; submucosa thickened
10	Nov. 3	8th	260	3	42	K.	—	Normal; not sectioned
11	Nov. 6	9th	320	5	90	D.	+++	Necrosis of mucosa; few amebas; greatly thickened submucosa
12	Nov. 6	9th	280	5	66	K.	+	Normal; not sectioned
13	Nov. 6	9th	340	5	18	D.	—	Not sectioned
14	Nov. 8	13th	800	5	42	K.	+++	Necrosis of mucosa; diphtheric membrane; few amebas; thickened submucosa
15	Nov. 8	13th	740	5	42	K.	—	Normal; not sectioned
16	Nov. 13	15th	920	5	46	K.	—	Normal; not sectioned
17	Nov. 13	15th	800	5	46	K.	—	Normal; not sectioned
49	Dec. 20	30th	400	5	65	D.	+++	Necrotic mucosa; thickened submucosa
50	Dec. 20	30th	400	5	91	K.	—	Normal

* No. 1 was treated exactly as were the others except that amebas were not injected.

medium. For the substrain used in the experiments listed in table 1, the medium was enriched with rice starch. The kittens were purchased on the streets. Well established methods of this laboratory were used in feeding and caring for them. Sanders[26] described these methods in detail.

As stated, one of the objectives of the experiments was to secure for study very early lesions in the mucosa of the colon. The method of laparotomy referred to was used for the experiments summarized in tables 1 and 2. After exposure of the colon, the latter was ligated near the rectum. By means of a Luer syringe, the culture fluid which contained amebas as well as yeasts and

26. Sanders, E. P.: The Effects of Infection with Endamoeba Histolytica on the Cells in the Blood of Kittens; in manuscript, 1928.

bacteria was injected through a no. 1 hypodermic needle. The shock of the operation lowered the resistance of the kitten and the ligature prevented the voiding of the material, so that the amebas had a better chance of establishing themselves than when introduced into the open bowel. I [18] had demonstrated previously that the starch-fed amebas, referred to heretofore, are infective and pathogenic in kittens when the method of laparotomy is used. An objection

TABLE 2.—*Experiments with Laparotomy. (The Endamebas Inoculated Were Cultured in Boeck & Drbohlav's Medium Without Starch.)*

Kitten	Date of Operation	Kitten's Weight, Gm.	Fluid Injected, Cc.	Lapse of Time, Hrs.	Cat Died (D.) Cat Killed (K.)	Degree of Infection	Character of the Lesions
18	Nov. 17	260	5	22	K.	+	No lesions showing amebas; colon poorly fixed
19	Nov. 17	280	5	20	K.	+	No lesions; no amebas in adherent mucus; colon poorly fixed
20	Nov. 17	250	5	18	K.	—	Colon was normal
21	Nov. 18	840	5	42	K.	+++	Generalized necrosis of mucosa; inflammation and thickening of submucosa
22	Nov. 18	920	5	18	D.	+	Not sectioned; appeared normal
23	Nov. 20	640	5	24	D.	+	Localized lesions in the colon contained living amebas
24	Nov. 22	1,200	5	25	K.	++	Slight necrosis; no amebic lesions
25	Nov. 21	1,250	5	26	K.	—	Normal
26	Nov. 22	1,500	5	18	D.	—	Not sectioned; appeared normal
31	Nov. 29	500	4	48	D.	—	Not sectioned
41	Dec. 6	1,260	5	72	K.	+	Microscopic lesions near valve and ligature
45	Dec. 6	1,100	5	73	K.	—	Appeared normal; not sectioned
51	Jan. 14	450	5	90	K.	++	Small ulcer in cecum, otherwise no lesions
52	Jan. 26	475	5	54	K.	++	Picture similar to that in kitten 51, except that the lesion in the cecum was smaller
59	Feb. 8	800	5	48	K.	—	Normal
61	Jan. 21	500	5	48	D.	—	Not sectioned
62	Jan. 21	520	5	72	D.	+	Several ulcers
63	Feb. 15	475	5	92	K.	—	Normal
64	Feb. 15	500	5	42	K.	—	Normal
65	Feb. 21	325	5	44	K.	—	Normal
66	Feb. 21	280	5	44	K.	—	Normal
67	Feb. 21	375	5	44	K.	—	Normal
68	Feb. 21	375	5	44	K.	—	Normal
69	Feb. 25	450	5	..	D.	—	Accidently killed during operation
70	Feb. 25	500	5	46	K.	++	One microscopic ulcer in cecum, otherwise normal
75	Feb. 28	520	5	45	K.	—	Normal
76	Feb. 28	520	5	45	K.	+++	One microscopic lesion near ileocecal valve, otherwise normal
77	Feb. 29	750	5	64	K.	+	Several small lesions; all contained amebas
78	Mar. 8	450	5	42	D.	—	Not sectioned
79	Mar. 8	450	5	26	K.	++++	Complete destruction of mucosa; colon invested with diphtheric membrane
84	Mar. 20	700	5	46	K.	—	Normal
85	Mar. 20	750	5	46	K.	+	
87	Mar. 24	700	5	72	K.	—	Normal

to this method was that pathologic conditions were induced by the operation per se independent of the lesions of amebiasis. Ample controls were maintained to demonstrate that the lesions resulting from the operation were not of a character to render the method unsuitable.

Infection of kittens was also accomplished by rectal injection. This method, well described in the literature, consisted of injecting the material by way of the anus through a rubber catheter into the colon. The anus was not subsequently closed. This procedure did not appreciably disturb the kitten or prevent the normal functioning of the bowel. Symptoms of dysentery

TABLE 3.—*Experiments with Rectal Injection. (The Endamebas Injected Were Cultured in Boeck & Drbohlav's Medium Without Starch.)*

Kitten	Kitten's Weight, Gm.	Dates of Injections*	Probable Prepatent Period, Days	Symptoms	Date on Which Kitten Died (D.), Was Killed (K.) or Experiment Was Discontinued (E.D.)	Degree of Infection	Character of Lesions
27	2,500	Nov. 22, 26, 28, Dec. 2, 9.....	..	.....	Dec. 23, E.D.	..	Animal remained healthy
28	1,380	Nov. 25, 28, 30	..	.....	Dec. 3, E.D.		
30	400	Oct. 10, 14, 19, 29, Nov. 11 (P. Nov. 16)	5	Bloody mucus	Nov. 26, K.	—	Many masses adherent to mucosa; sections showed superficial necrosis; no amebas
31	500	Nov. 29 (Dec. 3 N.), laparotomy	..	.....	See table 2		
32	400	Nov. 29.....	..	Bloody mucus	Nov. 30, D.	—	Bloody mucus in the jejunum; bacillary dysentery
33	640	Dec. 1, 8.....	5	Bloody mucus, Dec. 13	Dec. 16, K.	+	Lesions distributed throughout the colon
34	540	Dec. 1.....	..	.....	Dec. 9, D.	—	Not sectioned because of autolytic changes
35	560	Oct. 13, 14, Nov. 17 (P. 19)...	2	.....	Dec. 2, K.	+	Mucus patches adherent to mucosa; amebas not present in tissues
36	.....	Dec. 2.....	..	.....	Dec. 3, D.	—	
37	440	Dec. 2, 8.....	..	.....	Dec. 12, D.	—	
38	400	Dec. 2.....	..	.....	Dec. 4, D.	—	Pneumonia
39	430	Dec. 2.....	..	.....	Dec. 5, D.	..	Pneumonia
40	1,120	Dec. 5.....	..	Bloody mucus, Dec. 17	Dec. 20, D.	—	Bloody mucus in the jejunum
42	1,300	Dec. 5.....	..	.....	Dec. 12, E.D.		
43	1,200	Dec. 5.....	..	.....	Dec. 12, E.D.		
44	650	Dec. 5, 6.....	..	Bloody mucus, Dec. 13	Dec. 20, D.	—	Bloody mucus in the jejunum
46	1,380	Dec. 5.....	..	.....	Dec. 15, E.D.		
48	540	Dec. 9.....	..	.....	Dec. 12, D.	—	Pneumonia
55	1,200	Jan. 14.....	..	.....	Jan. 15, D.	—	Pneumonia
56	900	Jan. 14, 19.....	..	Bloody mucus, Jan. 21	Jan. 21, D.	—	Bacillary dysentery
57	950	Jan. 14, 18 (F.).....	..	Bloody mucus, Jan. 21	Jan. 21, D.	..	Bloody mucus in jejunum
58	875	Jan. 14, 19 (F.).....	..	.....	Jan. 21, D.	..	Peritonitis
71	275	Feb. 21, 27 (F.) (P. Feb. 23).	1	Bloody mucus	Mar. 5, K.	+++	Many shallow inflammatory lesions; many amebas in lesions
72	275	Feb. 21, 23, 27 F. (P. Mar. 1)	3	.....	Mar. 2, K.	+	Lesions containing amebas not observable
73	250	Feb. 21, 23, 27 F. (P. Feb. 29)	2	Bloody mucus	Mar. 3, K.	+++	Few lesions, much inflammation
74	275	Feb. 21, 23, 27 F.....	..	.....	Mar. 2, K.	—	Sections showed lesions containing E. histolytica
78	450	Mar. 2, 4, 6 (N. 8).....	..	.....	See table 2	..	Table 2
79	450	Mar. 2, 4, 6 (N. 8).....	..	.....	See table 2	..	Table 2
80	400	Mar. 6, 12, 19 F. (P. Mar. 22)	3	Bloody mucus	Apr. 13, D.	+++	Many lesions; much necrosis; many amebas in lesions
81	400	Mar. 6, 12, 19, 22 F. (P. Mar. 24)	2	.....	Apr. 25, K.	+++	Multiple lesions general in distribution
82	400	Mar. 6, 12, 19, 22 F. (P. Mar. 24)	2	Bloody mucus	Apr. 9, D.	..	Sent to Kansas State University while infected
83	700	Mar. 8, 12, 19, 22 F. (P. Mar. 27)	5	.....	Mar. 27, K.	++	Multiple lesions
84	700	Mar. 8, 9 F., 12 (Mar. 20 N.)..	..	.....	Mar. 20, see table 2	..	See table 2
85	750	Mar. 8, 22 (20 N.).....	..	.....	Mar. 20, see table 2	..	See table 2
86	400	Mar. 19, 22 F. (29 N.).....	..	.....	Mar. 29, K.	..	Colon lost
88	600	Mar. 27 F., 29 F. (P. Apr. 3).	5	Bloody mucus	Apr. 10, K.	+++	Multiple lesions
89	600	Mar. 27 F., 29 F. (P. Apr. 2).	4	Bloody mucus	Apr. 10, D.	+++	Multiple lesions; few amebas in lesions

* Key: P. = positive, N. = negative, F. = feces from an infected kitten.

always occurred in such kittens by the time that the fecal diagnosis was positive. Table 3 shows that the animals were kept from four to thirty-four days; when killed, all of them showed lesions. Some of these were as small as the smallest detectable lesions resulting from laparotomy. Both sorts are described in the following matter under appropriate headings.

One of the greatest difficulties was to locate the earliest lesions. Little time could be given to the examination of the fresh colon because autolytic changes set in quickly. One method finally devised was that of spreading the opened colon out in a petri dish, fixing it in hot Bouin's fluid for about half an hour or longer, then pouring the latter off and adding 70 per cent alcohol. There was a hemorrhagic halo surrounding even the tiniest lesion, and the yellow color given by the picric acid caused this halo to stand out clearly when the tissue was examined under the binocular microscope. Another method was that of sectioning the entire colon. Only a small portion of the ribbon could be mounted, but the rest was preserved between sheets of paper, which were carefully labeled. Cedar oil was used for clearing, and the sections were stained with iron hematoxylin and counterstained with eosin.

GENERAL ANALYSIS OF THE EXPERIMENTAL DATA

Eighty-four kittens were used, and fifty-eight were of experimental value for this problem. Thirteen died, either of bacillary dysentery or pneumonia or a complication of these diseases, during the latter part of November and December and January (table 3). These data indicated that kittens available from the streets in this climate during the aforesaid months are of lowered vitality and hence that other seasons of the year should be selected. Five kittens were given over to other work because they had evidently developed the resistance that comes with age. The data here presented agreed with those of other workers that unweaned kittens are also resistant to amebic infection. Kittens 65, 66, 67 and 68 were unweaned and all were negative after laparotomy. I was unable to infect eight other unweaned kittens, not listed in the tables, by the method of rectal injection. None of the observed phenomena indicated any cause for the resistance of the older kittens, but there was evidence that something in the mother's milk protected suckling kittens from experimental amebiasis, as well as from other infections. Kittens 71, 72, 73 and 74 were weaned in the laboratory. They soon became infected after the change of diet.

The colons of eight of the kittens that died during the night were not sectioned because of autolytic changes.

Of forty-nine kittens inoculated after laparotomy, twenty-three gave positive reactions. In nos. 2, 3, 11 and 14, table 1, and in kitten 79, table 2, the lesions were severe. The entire mucosa was necrotic and teeming with endamebas, and constituted a diphtheric membrane. In kittens 2 and 3, table 1, the submucosa was also invaded. The zone nearest the ligature was most severely affected. But in nos. 51, 52, 70, 76 and 77, the only lesions discernible occurred either in the cecum or near the ileocecal valve. In kitten 41, there were small lesions near the

ileocecal valve and near the ligature as well. Extremely small lesions were demonstrated in kitten 41 (after seventy-two hours), kitten 70 (after forty-six hours) and kitten 76 (after forty-two hours). It was impossible to find amebic lesions at autopsy in eight of these that were infected or to demonstrate them in sections. A detailed description of the lesions with figures and a discussion is reserved for the following paragraphs.

Five of the twenty-six kittens of table 1 and table 2, found not to be infected at autopsy, died during the night. The colons of all the others appeared normal by macroscopic study. Tissues from the colons of fifteen were sectioned and studied microscopically. The vascular areas were somewhat hyperemic; but whenever conditions permitted the fixation of the colon immediately at death, the mucosa was normal. Number 87 of table 2 was kept seventy-two hours, no. 63 of table 2 ninety-two hours and no. 50 of table 1 ninety-one hours before autopsy, and in these as well as in most of the others, necrosis was not demonstrated. Figure 2 is a camera lucida drawing of the mucosa of the colon of kitten 50. All the kittens that were not infected, save kitten 1, received the same inoculum as those that were, as stated in the foregoing paragraphs; this material contained the amebas with an accompanying flora of yeasts and bacteria. The kittens that were not infected were treated the same in every other respect as those that were infected. It is therefore demonstrated that the lesions in the latter were due primarily to injury inflicted by *Endameba histolytica* and not to the operative procedure or to bacteria.

With four exceptions, nos. 30, 31, 33 and 35 of table 3, those treated by rectal injection, during November, December and January, were either age resistant or died before the experiments were concluded. Fifteen, all small kittens, were given injections during February and March; ten showed infection (table 3). Kitten 79 was counted infected because, despite a negative fecal diagnosis, it was later used for laparotomy and a diphtheric membrane developed in the colon in twenty-six hours, while the sister kitten treated likewise remained uninfected. It will be noted from table 3 that when fecal material from an infected kitten was used, infection followed much more often than after the use of amebas in culture fluid. This is evidence additional to that I [18] gave in a previous article that the chief factor in infectivity is the adjustment of the amebas to the environmental conditions in the colon of the kitten.

In none of the group listed in table 3 was the submucosa of the colon invaded. The lesions were usually widely distributed but were small, leaving the greater part of the mucosa still functional. The region about the ileocecal valve was the most severely injured in every case. Most investigators have reported death usually occurring in

kittens within from four to nine days after the end of the prepatent period. Especial reference may be made to Dale and Dobell (1917). But in this work no. 80 lived twenty-four days and no. 81, thirty-four days. All the others, except kitten 82, were killed though some were in a comatose condition. Number 82, which had withstood transfer to Kansas City while infected, lived sixteen days.

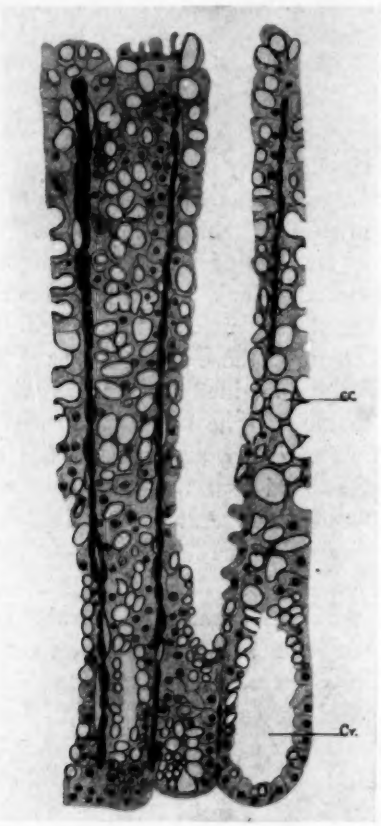

Fig. 1.—Camera lucida drawing of a section of the mucosa of a dysenteric colon of kitten 83 showing general enlargement of the crypts (*Cr.*) and goblet cells (*G. C.*); × 350.

DESCRIPTION OF THE LESIONS

In the unobstructed bowel the first symptom of intestinal amebiasis in the kitten was diarrhea. A copious secretion of the glands of the digestive tract must have occurred to produce the semifluid feces. The slides of the infected colons were therefore carefully studied and compared with those of the colons not infected in search of anatomic evidence of this hypersecretion. Figure 1 is a camera lucida drawing of

the mucosa of kitten 83, which was affected about seven days with dysentery (table 3). The crypts, it may be noted, are opened wide and the goblet cells are prominent, indicating that they were secreting actively when the kitten was killed. Sections through the colon of kitten 30 (table 3) showed the same characteristics as the tissue represented in figure 1, but lesions containing amebas could not be found. This kitten was heavily infected on November 16 and for at least a week thereafter, but infection was not present at autopsy on November 26. A similar condition was found in the colon of kitten 35 (table 3), although the latter did not show amebiasis at autopsy. Figure 2 is drawn from a

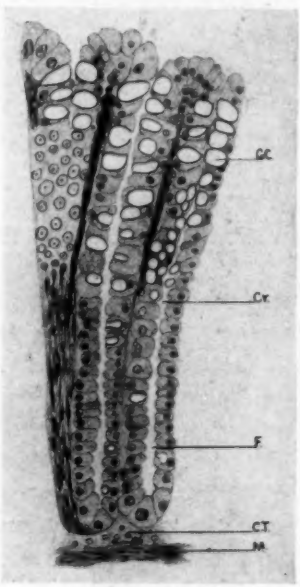

Fig. 2.—Camera lucida drawing of a section of the mucosa of the colon of kitten 50 killed ninety-one hours after inoculation by laparotomy and found negative for *Endameba histolytica;* × 350.

Abbreviations: *Cr.*, crypt; *C. T.*, connective tissue; *G. C.*, goblet cell; *F.*, fundus of the gland; *M.*, muscularis mucosa.

section of the mucosa of kitten 50 (table 2). This animal was kept ninety-one hours after a laparotomy, but amebas were not found. Evidences of dysentery in this colon were lacking; the mucosa appeared normal. In general, the sections showed that the condition represented in figure 1—that is, widely opened crypts and distended goblet cells—is a common characteristic of amebic dysentery in the kitten. In the early stages of the amebiasis, ulcers, if present were small—one had to search for them.

It was instructive to study the ulcers under the binocular microscope immediately after removal of the colon, while the latter was still living. To accomplish this, the colon was opened up in warm Locke's solution or normal sodium chloride, and the fecal material carefully washed away. The organ was then spread out in a petri dish in the same solution. The lesions appeared as hyperemic areas. When a bit of this tissue was teased out and mounted on a slide, it was seen under the compound microscope to contain numerous amebas. These were gorged with red blood cells and were in active locomotion. Therefore, some sort of injury had been inflicted which damaged the mucosa causing bleeding. The amebas profited by this injury, gaining a food supply of red blood cells.

Stained sections through ulcers containing amebas in twenty-four kittens were studied in several hundred slides. The general character of these lesions is indicated in tables 1, 2 and 3. It required over forty hours for the amebas to produce detectable ulcers after inoculation into a kitten by laparotomy. Ulcers could not be found in four kittens that were positive when killed after from twenty to twenty-seven hours, though in two of these cases, nos, 18 and 24, the entire colon was sectioned. No differences were observed between the early ulcers of six kittens infected by laparotomy and nine infected by rectal injection. In some of these early lesions, the epithelial cells of the mucosa were, for the most part, still intact (figs. 3, 6 and 7).

Figures 3, 4 and 5 are drawn from the colon of kitten 83, which was killed at the end of a five day prepatent [27] period following rectal injection. Figure 3 represents the entire lesion, while figure 5 shows in greater detail the group of amebas. Figure 4 is a diagram which indicates the relationship of the lesion to the mucosa as a whole, to the other tissues of the colon and to the lumen of the latter. In figure 3, it may be noted that there are two areas in which the cells of the lining mucous membrane are necrotic, also that the goblet glands are exceedingly prominent in adjacent cells that appear to be on the road to necrosis. Mucus and plasma appear to have enveloped and injured the attacking amebas. In the zone of necrosis deepest within the fold, there are evidences of an inflammatory reaction. Figure 5 shows the details of the nuclei of the amebas, some of which appear to be necrotic and others to be undergoing necrosis. The cytoplasm also appears vacuolated to an unusual degree.

Portions of early ulcers are pictured in figures 6 and 7. Figure 6 is a drawing from the outer margin of the only lesion that could be found

27. The "prepatent" period extends from the time the infective parasites enter the body of the host until their offspring can be recovered by specified laboratory methods. This period usually differs from the incubation period; the latter involves symptoms and is ordinarily longer.

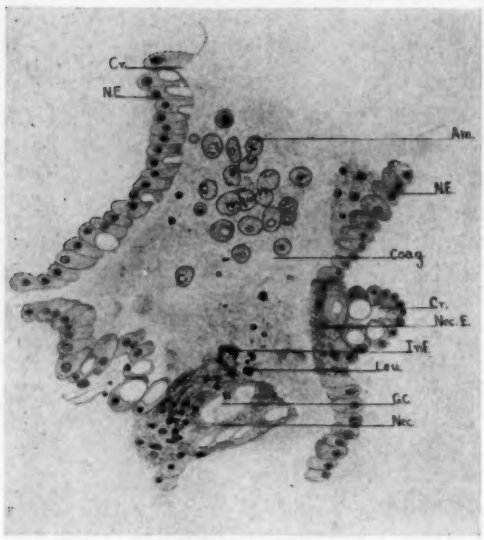

Fig. 3.—Camera lucida drawing showing a cluster of trophozoites of *E. histolytica* in a part of the lumen of the colon which is included within a fold of the mucosa. The outer layer of epithelial cells of the mucosa shows two necrotic areas; × 175.

Abbreviations (figs. 3 and 4): *Am.* amebas; *A. P.*, Auerbach's plexus; *Coag.*, plasma and mucus coagulated by the fixative; *Cr.*, crypt; *C. M.*, circular muscles; *G. C.*, goblet cell; *Inf.*, zone of inflammation; *L.*, lesion; *Leu.*, leukocyte; *L. M.*, longitudinal muscles; *M.*, mucosa; *N. E.*, normal epithelium; *Nec. E.*, necrotic epithelium; *Nec.*, necrotic area of the mucosa; *S.*, serosa.

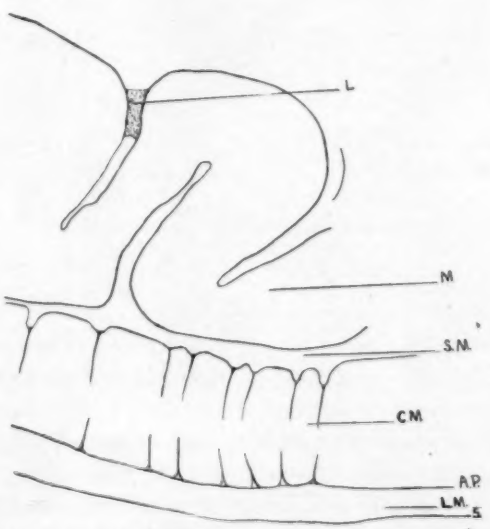

Fig. 4.—Outline of a section through the entire wall of the colon in which the lesion shown in figure 3 occurred, showing the location of the lesion and its relation to the other tissues of the colon; × 16.

in kitten 70, killed forty-six hours after a laparotomy. Necrosis of the epithelium is seen in the area beginning at the reader's left. Throughout the lesions, necrosis did not extend more than for several cell layers in depth. The amebas are enveloped in what appears to be a fluid containing much cellular debris. A number of them appear to be in contact with normal epithelial cells. Figure 7 pictures the beginning of an inflammatory reaction in a zone between two crypts that is made up largely of connective tissue. This drawing is from a lesion in kitten 76, killed forty-five hours after laparotomy.

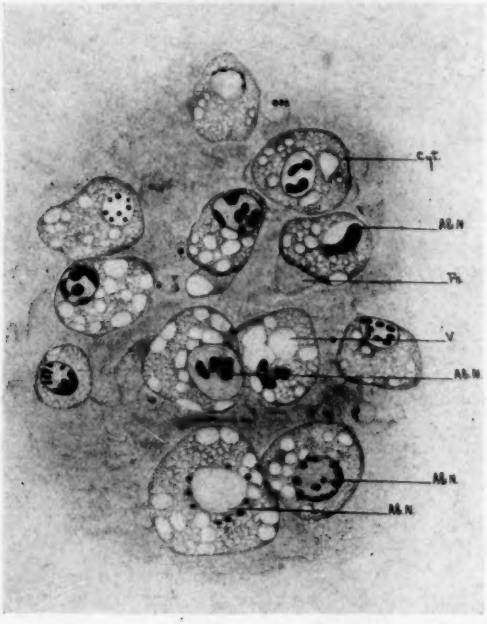

Fig. 5.—Camera lucida drawing of a portion of the lesion represented in figures 3 and 4, showing a group of *Endameba histolytica* with abnormal nuclei; × 800.

Abbreviations: *Ab. N.*, abnormal nuclei; *Cyt.*, cytoplasm; *Ps.*, pseudopodium; *V.*, vacuoles.

Figure 8 portrays an ulcer in the colon of kitten 41, killed seventy-two hours after laparotomy. In this lesion, the amebas were separated from intact epithelium by a zone of necrosis and plasma. It appears remarkable if so much destruction as is shown here was produced by the few amebas present. This ulcer occurred near the ligature that was tied around the colon near the rectum. Another ulcer, slightly more advanced, occurred in this colon near the ileocecal valve, but otherwise the mucosa was normal. It appears plausible that this ulcer may have been produced by a cytolytic toxin.

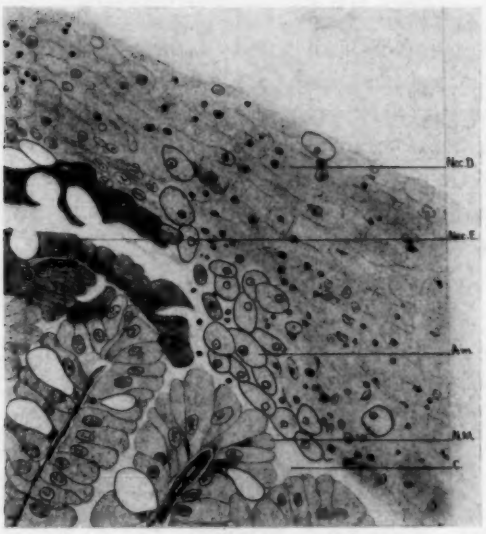

Fig. 6.—Camera lucida drawing of the outer margin of an ulcer in the colon of kitten 70; × 350.

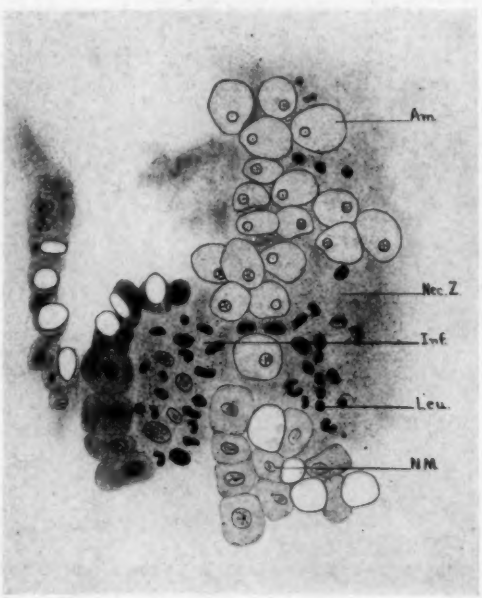

Fig. 7.—Camera lucida drawing of a portion of an ulcer in kitten 76; × 350. Abbreviations: *Am.*, amebas; *C.*, crypt; *Inf.*, zone of inflammation; *Leu.*, leukocytes; *Nec. Z.*, zone of necrosis; *Nec. E.*, necrotic epithelium; *N. M.*, normal mucosa.

Figure 9 was drawn from a section through a solitary lymph gland near the ileocecal valve of kitten 76 and illustrates what appeared to be an encounter between amebas and lymphocytes. A zone of necrosis separated the amebas from the normal cells within the gland.

A small section through a diphtheric membrane is represented in figure 10. The amebas had penetrated into the connective tissue between

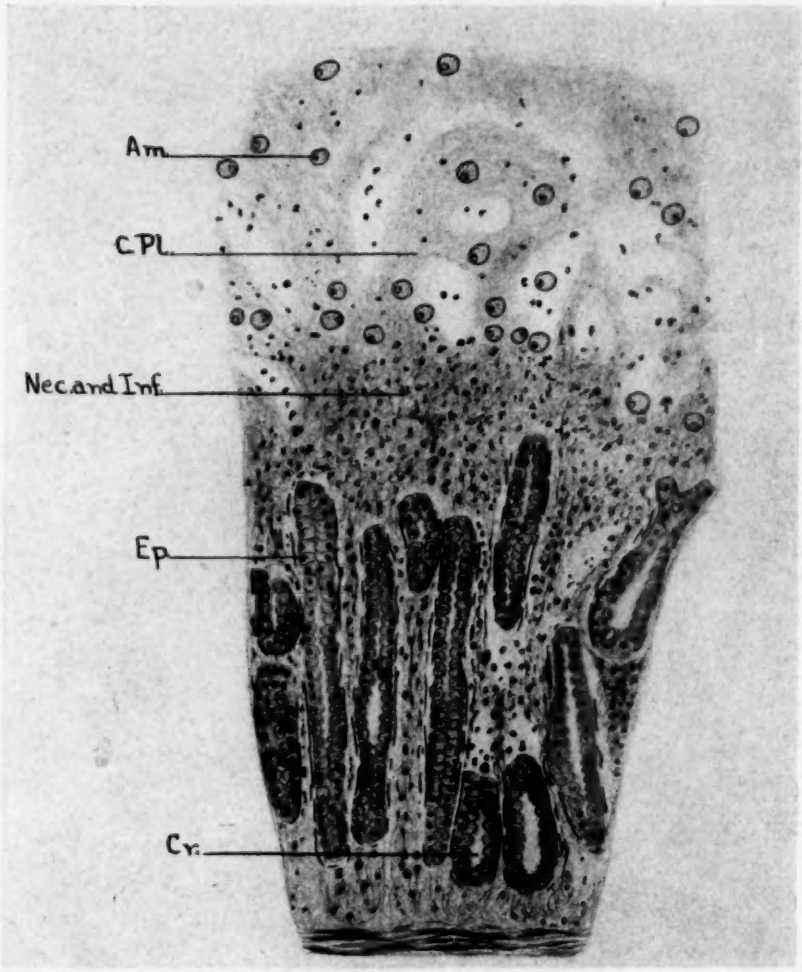

Fig. 8.—Camera lucida drawing of an ulcer of kitten 41; × 175. Abbreviations: *Am.*, amebas; *C. Pl.*, coagulated plasma; *Cr.*, crypt; *Ep.*, epithelium; *Nec. and Inf.*, zone of necrosis and inflammation.

the fundus of the glands and the muscularis mucosa and were seen in compact nests as though active multiplication had occurred there. The rest of the mucosa had almost completely disintegrated.

In all of the lesions studied, as well as in those figured and described, necrosis of tissue appeared to have commenced in the epithelium most

exposed to the fecal contents of the lumen. The lining epithelium of the crypts appeared normal in the early lesions, while cells not so situated appeared necrotic. A cytolytic toxin appeared to have been operative but conclusive evidence was not found that this toxin was excreted by the amebas.

It is not improbable, however, that the amebas may also have attacked the tissues by mechanical means. The work of Mast and Root,[28] who observed a free-living ameba cut a paramecium in two, indicated that ameboid organisms may engage in physical combat.

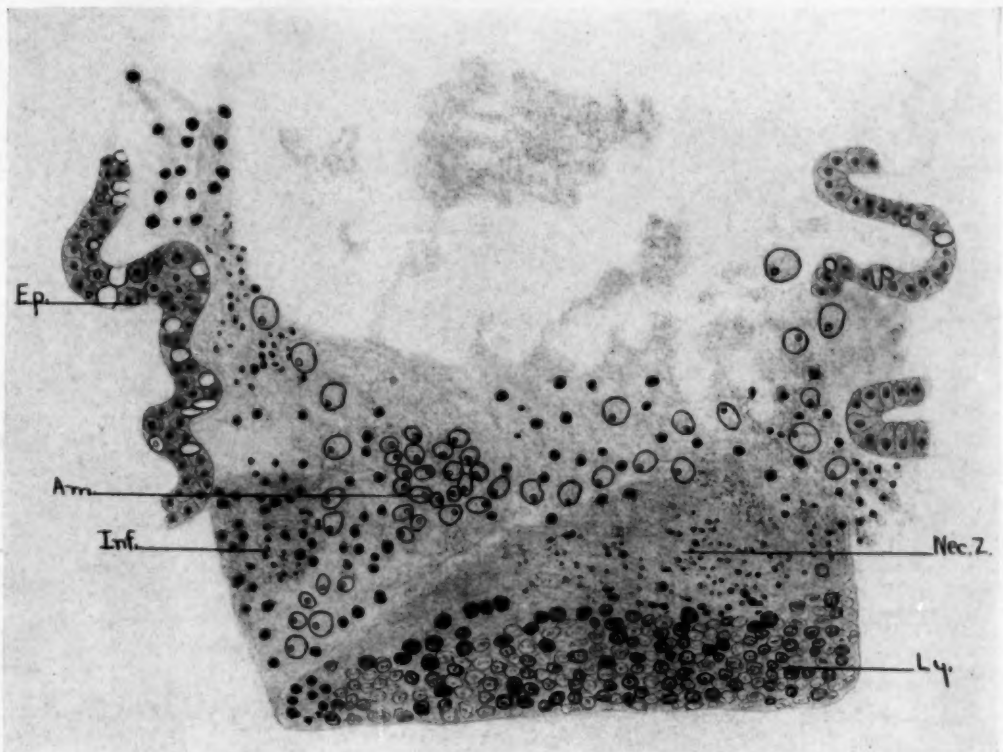

Fig. 9.—Camera lucida drawing of an amebic lesion in a solitary lymph gland of the colon of kitten 76 showing invasion of the gland by amebas; × 175.

Abbreviations: *Am.*, amebas; *Ep.*, epithelium; *Inf.*, zone of inflammation; *Ly.*, lymphocytes.

GENERAL COMMENT

As stated previously, the chief objective of this work is to determine the method by which *Endameba histolytica* inflicts injury on the tissues of the colon. Some of the difficulties of such a study were anticipated

28. Mast, S. O., and Root, F. M.: Observations on Amoebae Feeding on Rotifers, Nematodes and Ciliates, and Their Bearing on the Surface Tension Theory, J. Exper. Zool. **9**:301, 1908.

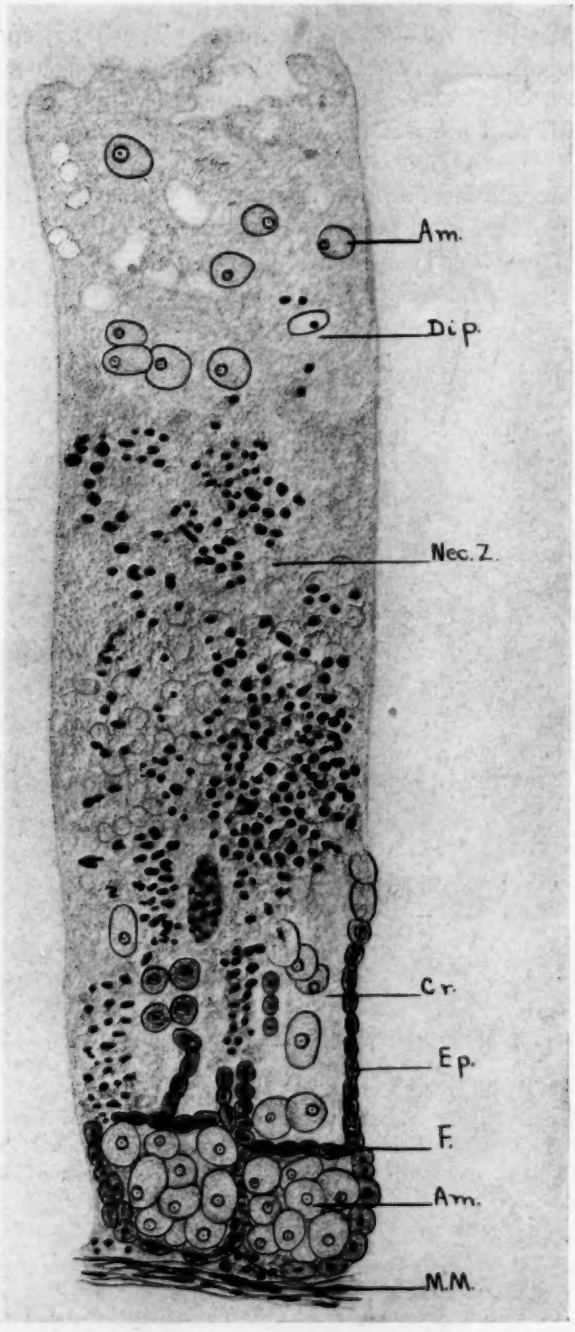

Fig. 10.—Camera lucida drawing through the mucosa of the colon of kitten 79 showing a diphtheric membrane; × 350.

Abbreviations: *Am.* amebas; *Cr.*, crypt; *Dip.*, diphtheric membrane; *Ep.*, remnants of epithelial cells; *F.*, fundus of the gland; *M. M.*, muscularis mucosa.

at the start, but as the work progressed many others appeared. The chief results thus far have been simplification of the problem by the surmounting of some of these difficulties. This will be made apparent by the discussion of the following questions: 1. Do the amebas multiply only within the lesions or is multiplication general throughout the entire fecal content of the infected bowel? 2. What are the steps in the process of the development of ulcers and are they the same whether in the areas first attacked or in other areas of the mucosa to which the infection spreads? 3. Does penetration of the tissue by the amebas continue after the death of the host, when the autopsy is not immediately performed? 4. What part in the production of lesions is played by the bacterial flora; that introduced with the ameba, as well as that already resident in the bowel?

Until the work of Boeck and Drbohlav,[20] it was generally held that multiplication of the amebas in the infected bowel occurred only within the tissues. But since the successful cultivation of this organism has been accomplished in vitro without the presence of tissue elements, several writers have suggested that multiplication may occur on the surface of the mucosa or even within the fecal content. From the viewpoint of pathogenesis, it is important to know where multiplication occurs, because it may be found that this factor influences the steps in the process of invasion of tissue and also the relation of these steps to the occurrence of symptoms. For example, if multiplication occurs only within the tissues, the following might be the sequence of events in those cases of infection which result from the ingestion of cysts. Only a few trophozoites would reach the colon, because not many cysts would be likely to be swallowed at one time. If conditions of survival required that the trophozoites attack the tissues at once, it is obvious that from one to several small lesions leading to ulcers would soon be produced. From these ulcers, the parasites would spread to attack other areas of the mucosa. When the lesions became sufficiently numerous and sufficiently severe, symptoms of dysentery would occur. According to this view, dysentery is a reaction of the host which follows ulceration due to injuries inflicted by the parasites.

On the other hand, if multiplication occurs also on the surface of the mucosa or in the fecal material within the lumen, the following might be the course of events. The trophozoites would multiply rapidly and, as is explained in detail in the foregoing paragraphs, soon would become sufficiently numerous to stimulate the secretory tissues of the bowel, producing diarrhea. Tissue invasion by the amebas would then occur and, as a result of the discharge of material from the ulcers, the symptoms would become those of dysentery.

I believe that in the kittens studied multiplication of the amebas occurred within the lumen in the fecal material independent of the

ulcers. As evidence, I cite the following observations: 1. As stated in the review of the literature, *E. histolytica* thrives when cultivated in vitro even to the completion of its life cycle; therefore, access to living tissue is not obligatory. 2. Ingestion of starch causes it to grow and divide much more rapidly than it does when this food is not available. Therefore, the nutritive conditions for optimal growth do not necessarily involve living tissue. 3. Within the lesions the reaction of the host appeared to kill the amebas or at least to injure them so that multiplication was retarded (figs. 3 and 5). 4. Diarrhea appeared in all the kittens infected by rectal injection as soon as the fecal diagnosis was positive, that is, at the end of the prepatent period. When kittens were killed at this time and examined microscopically, there did not appear to be enough ulcers to serve as a multiplication ground for the enormous numbers of trophozoites occurring in the feces. 5. In the sections, there was evidence of multiplication of the amebas within the lesions (fig. 10), but there was not any indication that necrotic material containing amebas had been discharged into the lumen from any of these early lesions. Since the feces were usually teeming with the organisms, it did not appear probable that they could all have been derived from material discharged from the ulcers. 6. Kittens 30 and 35 were affected for ten and thirteen days, respectively, with amebiasis, and yet no lesions containing amebas could be found in sections of these colons. On the other hand, the following facts indicate the need of caution before committing oneself definitely concerning the multiplication of these parasites. The kittens referred to were infected by rectal injection and not by the natural means of ingesting cysts. The five cubic centimeters of fluid usually injected were enough to fill half of the colon of such young kittens and no doubt contained many more amebas than would reach the colon at one time if the animal ingested the cysts. Furthermore, fecal diagnosis was negative in kittens 33, 74 and 79 of table 3, and yet the colons of the first two were found to contain amebas at autopsy, while the latter must have been infected because of the diphtheric membrane which developed in twenty-six hours following laparotomy (table 2). These observations satisfied the requirements for the view that multiplication occurs only within the lesions. Then, again, diarrhea and sometimes dysentery occurred in some of those kittens that were not infected with amebas. This fact indicated that other agencies may have operated also in the kittens that were found infected. Hence the need of further research on this question. Should it be definitely established that the amebas multiply within the lumen and that diarrhea precedes ulceration of the colon, a strong case will have been built up for the theory that the amebas excrete a toxic substance. It appears improbable that without such excretion their mere presence in the bowel would excite the glands to an extent necessary to cause this symptom.

The second question stated in the foregoing part of this article concerns the steps in the process of the production of ulcers. Wenyon [18] described these steps in the cat as follows:

> In sections of the ulcerated gut it is easy to study the invasive process. The amoebae make their way to the bottom of the tubular gland in the large intestine. There they multiply and by pressure of their number or by the exertions of their pseudopodia and probably through some toxic substance excreted by them, the lining cells are weakened and separated and the amoebae pass into the connective tissue beneath. The epithelial cells commence to degenerate and the earliest stage of ulceration is reached. Bacteria are thus admitted and the destruction becomes more rapid while the amoebae push on to deeper layers and give rise to the characteristic undermined ulcers.

The data on which this author's statements were based had been obtained from observation of a series of kittens, of which six became infected with amebic dysentery. Three died during the night, and three were killed. Figures were not given to illustrate the successive steps in the process described. Wenyon [21] expressed the same opinion concerning human amebiasis, and stated further that human colons at autopsy "show every stage in the production of ulcers from the smallest yellow nodules to the large undermined ulcers." According to this view, the invasive process is the same whether studied in human beings or in kittens. As Wenyon speaks of undermined ulcers in the kittens which he studied, the lesions were much farther advanced than those of the present series. Careful drawings and descriptions presented by myself in the foregoing pages are evidence that in the kitten the steps in the invasive process are at least not always as described by Wenyon. A gland was found occasionally to be occluded in the colons of kittens 33, 83, 88 and 89, infected by rectal injection. Such glands appeared greatly distended by the continued secretion of mucus, and the epithelial cells appeared to have been flattened by the pressure thus produced. Some of these enlarged glands contained leukocytes, but amebas could not be found in any of them. But the glands were found almost always to be opened out toward the lumen rather than occluded, and the secretion of mucus appeared as the most marked characteristic of the reaction of the host. This altered condition of the crypts and the outflow of mucus may have prevented the migration of the amebas into these glands, particularly after dysentery had appeared. Necrosis of tissue always commenced in the epithelium outside the crypts. The inflammatory reaction became manifest in the connective tissue between the crypts, and there was evidence of an outflow of plasma and an outwandering of leukocytes. Necrosis continued until the entire mucosa became involved in the ulcer.

Concerning question 3, I observed a phenomenon which is here termed postmortem penetration; that is, continued invasion of the tissues

of the host, after the death of the latter, by the amebas. Kitten 23 of table 2 died during the night as a result of a poorly conducted operation. That it had been dead some time when autopsy was performed twenty-four hours after the operation was indicated by the temperature of the body, the condition of the colon and other organs and by the fact that though there were many active amebas in several hemorrhagic ulcers, none were present in the feces within the colon. The ulcers were about 2 mm. in diameter—as large as many of those found regularly in kittens killed several weeks after the close of the prepatent period following rectal injection. It appears remarkable that such large ulcers should have been produced solely by the amebas in so short a time. They were not present, however, at the time of the operation or they would have been observed, since at autopsy they were plainly visible in the wall of the colon before it was opened. Therefore it appears that, in this case, the amebas were able to penetrate the tissue after the death of the host much more rapidly than while the latter was living. Similar ulcers were seen in the colon of kitten 62 of table 2, but this animal died during the third twenty-four hour period following the operation. Scott,[29] working with *Balantidium* in the guinea-pig, noted that this ciliated protozoan may penetrate the mucosa of the cecum after the death of the host. She did not furnish evidence that such penetration occurs during the life of the latter.

As is well known, autopsies on human beings are seldom conducted immediately after death; frequently there is a lapse of from twenty-four to seventy-two hours. Councilman and Lafleur [23] (1891) and Wenyon [21] (1925) reported that amebas were usually found living in the tissues at the end of such periods. Dobell and Laidlaw [17] showed that *E. histolytica* might remain living in vitro at room temperature for three days. But cooling occurs more slowly in human beings than in kittens, so that in the former there is a considerable period during which the amebas may work at a temperature near the optimum. It is not improbable that under such conditions, without having to combat the resistance of the host, the parasites may penetrate rapidly.

It seems clear from the foregoing discussion that the procedure I adopted and the precautions I took were necessary. Briefly summarized, these were: (1) to study the symptoms of infected kittens and to consider them in connection with the lesions found at autopsy, (2) to use several methods of infecting kittens and to compare the kittens with each other and with controls, (3) to kill the kittens in the earliest stages of infection as well as in later stages and thus secure a series as complete as possible from the earliest lesions to well developed ulcers, (4) to

29. Scott, M. J.: Studies on the *Balantidium* from the Guinea Pig, J. Morphol. & Physiol. **44**:417, 1927.

study the lesions while the colon was still living and also in the freshly fixed colon as well as in the sections, (5) to kill tissues for sectioning with fixing fluids; but if this were impossible, to fix them as quickly after death as possible, (6) to study the mucosa of the entire colon, that already invaded by the amebas as well as that not so invaded. It will be granted that whether or not the steps in the invasive process are the same in human beings as in kittens, the weapons of attack of the amebas are always the same. I hold that pathogenesis may be much more satisfactorily studied in the kitten, because the experimental method may be applied. Furthermore, I believe that a clearer picture may be obtained from kittens killed in the early stages of amebiasis than from sections which have advanced to the stage of undermined ulcers with overhanging margins.

Question 4 concerns the complications caused by bacteria, which are always present with the amebas in the cultures and in the colon to which they are introduced. Thus far, little attention has been paid to a study of this factor, though its importance is realized. It is well known that the healthy bowel is not affected by the bacteria commonly living within the feces. It has been amply demonstrated by earlier workers as well as in this work that the bacteria commonly found associated with the amebas do not produce lesions when introduced without amebas into the colon. In this work, such lesions did not occur when amebas were introduced with the bacteria unless the protozoan parasites established themselves. But bacteria do invade all wounds that are accessible to them, and an inflammatory reaction occurs wherever a wounded surface is left exposed to their attack. Cytolytic products are produced, and necrosis of the tissue occurs. In the lesions studied and illustrated in this article, an inflammatory reaction was always manifest. According to most authorities on the pathology of amebiasis, inflammation is not one of the reactions of the body resulting from the form of injury inflicted by amebas. The best evidence for this belief is that amebic lesions of the liver, which do not contain bacteria, are not true abscesses; that is, the inflammatory reaction is absent.

It is obviously impossible to exclude bacteria from the colon of the kitten, and no one has thus far demonstrated that amebas free from bacteria can be grown in culture. Since the objective of this work is to determine what weapons the amebas use, the problem is seriously complicated by the bacteria. It has been impossible up to the present time to discriminate clearly between the results of bacterial activity and the results of amebic activity. Unless some method of doing so can be developed, it may be necessary to devise experiments involving tissue culture from which they can be excluded.

SUMMARY

1. This investigation was undertaken with the object of obtaining information on the methods employed by *Endameba histolytica* in its attacks on the tissues of the colon in kittens.

2. The kitten was chosen as the experimental animal because of its well known susceptibility to amebiasis.

3. The amebas were grown in culture in Boeck and Drbohlav's egg slant serum medium, and for the earlier experiments this medium was enriched by rice starch.

4. Two methods of infecting kittens were used: that of an injection of the material through the wall of the ligated colon following laparotomy, and that of rectal injection without a subsequent closing of the anus.

5. Eighty-four kittens were used and, from fifty-eight of these, data of value to this problem were obtained. Prepared slides of sections through amebic lesions were studied from fourteen kittens infected after laparotomy and from eight infected by rectal injection.

6. Amebic lesions could not be found in kittens that were proved infected after laparotomy when killed in less than forty hours after the operation, but lesions of great severity frequently were found after ninety hours, involving complete destruction of the mucosa and the investment of the colon by a diphtheric membrane.

7. When the amebas did not succeed in establishing themselves after laparotomy, the mucosa was normal up to ninety hours.

8. With the aid of the binocular microscope, minute lesions could be detected in a freshly fixed colon. Entire colons were frequently sectioned in an effort to find all the lesions.

9. The first ulcers that were detectable after laparotomy usually occurred near the ileocecal valve, and the lesions were always most severe in this area after rectal injection.

10. In kittens killed from four to thirty-four days following the injection of trophozoites through the anus, there were often well developed ulcers and also minute lesions, but the submucosa appeared not to be invaded in any case.

11. Infection followed much more frequently when feces from an infected kitten were injected than when amebas in culture were used.

12. The earliest observed symptom of intestinal amebiasis was diarrhea, which occurred at the close of the prepatent period (from two to five days). Evidences of anatomic alterations in the colon following diarrhea were found to consist in enlarged glands and pronounced goblet cells.

13. Ulcers in the colon were studied under the binocular microscope in Locke's solution while the tissue was still living. They appeared as hyperemic areas. Tissue was teased from these lesions and was found, under higher magnification, to contain living active amebas, which were suggestive of active tissue invaders.

14. In one colon, the invasion by the amebas of a solitary lymph gland was noted and figured.

15. The evidence of the lesions studied in this work does not support the view of several authorities that the initial attack of the amebas on the mucosa commences within the crypts, leading to the occlusion of the latter and the development of abscesses.

16. In all the material studied from kittens infected both after laparotomy and by rectal injection, the amebas appear to have begun the attack on the mucosa; that is, outside of the crypts. The ulcers appeared to be developed therefore by necrosis beginning at the surface, and not by the discharge into the lumen of necrotic material from abscesses.

17. The attack of the amebas appeared to have provoked a reaction of the host comprising hypersecretion of mucus and hyperemia. Other evidences of inflammation were also manifest, but the part played by bacteria in this reaction could not be ascertained.

18. There was evidence that the amebas were injured by the reaction of the host.

19. Although this investigation is still in progress, these data are published at this time because evidence heretofore presented has not included descriptions and figures of early lesions. Furthermore, the data support the view stated heretofore that the method of producing ulcers is different from that usually described.

CONCLUSIONS

In this investigation thus far, the nature of the attack of *Endameba histolytica* on the colon has not been definitely determined. This is due mainly to complicating factors which confuse the picture. But the results of the experiments suggest that the following events occur: The amebas multiply within the lumen of the colon and produce a toxin which causes the host to react by the hypersecretion of mucus, and diarrhea results. In various areas where conditions for the multiplication of the parasites have been most favorable, they attack the epithelial cells of the mucosa. The host responds by a more rapid secretion of mucus and plasma. These fluids envelop and apparently injure some of the amebas. But continued attack by amebas leads to an injury sufficient to cause bleeding. The amebas profit by this food and ingest the red blood cells. They continue to invade the tissue until an ulcer is formed,

extending to the muscularis mucosa. Beyond this point the infection was not followed in my material. The hypersecretion of the glands, the hyperemia and the rapid necrosis of tissue indicate the excretion by the amebas of a cytolytic toxin. In many of the lesions, the amebas were not in immediate contact with the cells but separated by a zone of necrosis. This also suggests their ability to inflict injury by means of toxins. Another method of attack may consist in the actual destruction of cells by physical means, as has been reported in the case of free-living amebas. This might result in injuries to the tissues that bring about bleeding. The general occurrence of bacteria in the lesions complicates the problem, because they may cause much of the necrosis that one could otherwise definitely attribute to the amebas.

METAPLASIA OF BASAL CELLS IN THE DUCTS OF THE PANCREAS: ITS CONSEQUENCES*

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THE PROBLEM OF METAPLASIA OF BASAL CELLS

When Krompecher[1] in his early investigations of tumors of the skin first recognized the fact that the "rodent ulcer" was clinically as well as histologically a special type of tumor, he created the term, "basal cell carcinoma." His work showed that these tumors of the skin grow slowly, metastasize rarely and then only late, and that they do not tend to recur after excision; also, that the cells which compose these tumors resemble those occurring in the malpighian layer of stratified epithelium of the skin, form solid cell groups and arrange themselves in a plexiform manner.

Further investigations by Krompecher[2] and others demonstrated that the basal cells which are found between the cylindrical cells and the membrana propria of glandular organs can proliferate in a similar manner and give rise to basal cell carcinoma. Krompecher demonstrated such alterations in the mucous membrane of the nose, bronchi, uterus, prostate, breast, larynx, intestine and parotid gland. He drew attention to the fact that basal cell tumors in internal organs differ from basal cell tumors of the skin in that the former behave more like malignant tumors.

Krompecher believed that basal cell carcinoma could result from the proliferation of basal cells. He regarded the latter as a preliminary to cancer. He thought that the toxins of different bacteria act as irritants on the basal cells of different mucous membranes and lead to their proliferation.

In many instances experimental investigations have confirmed the morphologic observations which Krompecher recorded. For example, it has been shown that the benign epithelial proliferation which occurs at an early stage in the experimental production of tar cancer, can later give rise to a really malignant tumor. In this way, a potential difference between a relatively benign carcinoma, such as "rodent ulcer," and a really malignant basal cell carcinoma was disproved.

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1. Krompecher: Der Basalzellenkrebs, Jena, Gustav Fischer, 1903.

2. Krompecher: Beitr. z. path. Anat. u. z. allg. Path. **44**:88, 1908; **65**: 79, 1919; **70**: 489, 1922; **72**: 163, 1924; **76**:113, 1927; Ztschr. f. Geburtsh. Gynäk. **81**: 299, 1919; Arch. f. Laryngol. & Rhinol. **31**:443, 1918.

Brusis [3] ligated the ducts of the submaxillary glands in rabbits and after fourteen weeks demonstrated proliferative changes in the ducts. Ribbert [4] observed in the parotid glands of rabbits, similarly treaed, changes even more striking and bearing a close resemblance to carcinoma. Löwenstein [5] reported basal cell changes in the duct of the parotid gland in a case of Mikulicz's disease, in tuberculosis and in status lymphaticus. Krompecher regarded the tumors composed of mixed types of cells in the parotid gland, previously known as myxoendothelioma, as myxobasalioma.

Kawamura [6] produced metaplasia of the bronchial epithelium experimentally by irritants. Goldzieher [7] found basal cell changes in the larger bronchi in a case of diphtheria, while Teutschländer [8] noted similar changes in the lungs of rats which had died from bronchopneumonia and in the lungs of a child who had died from measles. Askanazy [9] could demonstrate metaplasia of basal cells in the lower respiratory passages in thirty-eight of ninety cases of epidemic influenza, and he also observed such changes in two cases of typical pneumonia.

Szabó [10] noted metaplasia of basal cells in the prostate, and Wolf [11] in the ductus deferens following gonorrheal epididymitis.

Krompecher regarded the so-called carcinoids in the intestines as tumors which had resulted from metaplasia of the basal cells.

Krompecher's statement that a proliferation of basal cells in glandular organs could be a preliminary to cancer has been verified by the occurrence of basal cell carcinoma in those organs in which a proliferation of basal cells was also found. Thus von Meyenburg [12] reported a basal cell tumor of the trachea, Siegmund [13] three similar tumors of the lung and B. Meyer,[14] among others, a basal cell carcinoma of the right main bronchus, which developed in a patient 53 years of age, following influenza. Wutsuji [15] has observed such tumors in the gallbladder.

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3. Brusis: Dissertation, Marburg, 1903, cited by Krompecher: Beitr. z. path. Anat. u. z. allg. Path. **70**:499, 1922.

4. Ribbert: Verhandl. d. deutsch. path. Gesellsch. **6**:133, 1904.

5. Löwenstein: Frankfurt. Ztschr. f. Path. **4**:187, 1910.

6. Kawamura: Virchows Arch. f. path. Anat. **203**:420, 1911; Ijishinbum, Japan, 1908, p. 753.

7. Goldzieher: Centralbl f. allg. Path. u. path. Anat. **29**:506, 1918.

8. Teutschländer: Centralbl. f. allg. Path. u. Path. Anat. **30**:433, 1919.

9. Askanazy: Cor.-Bl. f. Schweiz. Aerzte **49**:75, 1919; cited, Centralbl. f. allg. Path. u. path. Anat. **30**:443, 1919.

10. Szabó: Magyar Ovrosi Arch. **26**:248, 1925.

11. Wolf: Virchows Arch. f. path. Anat. **228**:227, 1920.

12. von Meyenburg: Centralbl. f. allg. Path. u. path. Anat. **20**:130 and 578, 1919.

13. Siegmund: Virchows Arch. f. path. Anat. **236**:191, 1922.

14. Meyer: Frankfurt. Ztschr. f. Path. **271**:517, 1922.

15. Wutsuji, cited by Krompecher: Beitr. z. path. Anat. u. z. allg. Path. **76**: 113, 1927.

REVIEW OF THE LITERATURE ON THE PROLIFERATION OF BASAL CELLS IN THE DUCTS OF THE PANCREAS

Kawamura [6] found the lining epithelium of the duct of Wirsung and its middle size branches changed from cylindrical to multilayer epithelium in a patient 43 years old, who suffered from distomum spathulatum of the pancreas. Nunokawa [16] and Nakamura [17] recorded similar observations. Kawamura remarked that, although distomum spathulatum occurs simultaneously in the biliary ducts of the liver and in the ducts of the pancreas, he found alterations which he regarded as due to metaplasia of the basal cells only in the ducts of the pancreas. In his case as well as in that reported by Nunokawa, the pancreatic duct showed inflammatory changes and the pancreas a chronic pancreatitis.

Kölliker [18] stated that the epithelium of the larger pancreatic ducts is of the cylindrical type, and that the height of the cells in these ducts is from 13 to 18 microns or, in the smaller ducts, from 5 to 7 microns.

The transformation of the cylindrical epithelium of the pancreatic ducts to multilayer epithelium, in three cases, was reported by Oberling.[19] In every instance the patient was over 50 years old, and the alterations were associated with interstitial pancreatitis.

The largest number of cases of metaplasia of basal cells in the pancreas previously reported are those recorded by Priesel.[20] He examined 140 cases and observed metaplasia of basal cells in thirteen instances. From his work it is evident that metaplasia of basal cells in the ducts of the pancreas cannot be regarded as of rare occurrence, but is apparently present in somewhat less then 10 per cent of cases. Priesel stated that metaplasia of basal cells in the pancreas occurs in elderly people, because ten of his cases were in persons over 60 years of age.

In his series the pancreas in five instances was normal. In one case, the organ showed a carcinoma. This tumor, however, did not arise from proliferating epithelium of a duct. It was of the glandular type. In two cases the pancreas contained a cystadenoma. In three, lipomatosis was noted, twice with formation of cysts. The cysts in his case 12, were not related to metaplasia of basal cells, although in another case a connection between the proliferation of basal cells and the formation of cysts was accepted. The remaining two cases were diabetes. Priesel regarded the alteration in the ducts of the pancreas as prosoplasia.

Scholtz [21] reported one case of metaplasia of basal cells in the ducts of the pancreas. She stated that the cause of this alteration was

16. Nunokawa, cited by Kawamura (footnote 6).
17. Nakamura: Med. Wchnschr. zu Kyoto, Japan **5**:1, 1908.
18. Kölliker, cited by Kawamura: Virchows Arch. f. path. Anat. **203**:420, 1911.
19. Oberling: Bull. de l'Assoc. franç. p. l'étude du cancer, 1921, p. 155.
20. Priesel: Frankfurt. Ztschr. f. Path. **26**:453, 1922.
21. Scholtz: Virchows Arch. f. path. Anat. **247**:467, 1923.

unknown. Abdrachmanoff [22] and Herxheimer [23] reported similar cases. Weichselbaum,[24] in his studies on the regeneration of the islands of Langerhans, noted proliferative changes in the ducts of the pancreas; while Kyrle [25] and Weichselbaum observed such changes in the duct of Wirsung in animals shortly after the ducts had been ligated.

Israel [26] described a cancroid in the pancreas of a man aged 50, and Lewisohn [27] a similar condition in a man aged 67, in which the pancreatic duct beyond the point of obstruction was markedly dilated and the pancreatic tissue necrotic. Kawamura regarded cancroids of the pancreas, gallbladder and lungs as being of similar origin. Herxheimer [28] reported the combination of an adenocarcinoma and basal cell carcinoma of the pancreas. Cysts were noted in the tail of the pancreas. Askanazy [29] recorded two cases of basal cell carcinoma of the pancreas.

It is therefore surprising that Walz [30] should have stated that to the year 1926 basal cell tumor of the pancreas had not been reported.

AUTHORS' OBSERVATIONS ON METAPLASIA OF BASAL CELLS IN THE DUCTS OF THE PANCREAS

In order to determine the relative frequency of metaplasia of basal cells in the ducts of the pancreas, we examined a series of 160 pancreases. Fresh specimens were obtained from the Pathological Department of the Metropolitan Saint Stephen's Hospital, Budapest. At least five or six sections from the head, body and tail were examined, and usually from eight to ten or more.

Table 1 shows that a proliferation of basal cells had occurred in fourteen of the pancreases examined, which means in 8.75 per cent.

Our chief interest was directed to the pathologic consequences of metaplasia of basal cells in the pancreas, and to its cause.

General Observations.—The replacement of the cylindrical epithelium of the pancreatic ducts by basal cells was found especially in the middle size and smaller ducts. Here the basal cells were often seen to have proliferated in a circumscribed manner, forming little buds or pillows. These buds were usually composed of several layers of basal cells and

22. Abdrachmanoff: Thèse de Genève. Contribution à l'anatomie pathologique du pancréas diabetique, 1912.

23. Herxheimer: Gewebsmissbildungen in Schwalbes Handbuch, Jena, 1913.

24. Weichselbaum: Sitzungsb. d. Wiener Akad. d. Wissenchaft, 1908, pt. 3, p. 117; 1910, pt. 3, p. 119.

25. Kyrle: Arch. f. micr. Anat. **72**:141, 1908.

26. Israel: Berl. klin. Wchnschr. **33**:45, 1896.

27. Lewisohn: Ztschr. f. Krebsforsch. **3**:528, 1905.

28. Herxheimer: Beitr. z. path. Anat. u. z. allg. Path. **41**:348, 1907.

29. Askanazy, cited by Priesel: Frankfurt. Ztschr. f. Path. **26**:513, 1922.

30. Walz: Centralbl. f. allg. Path. u. path. Anat. **37**:748, 1926.

in some instances were partly covered by cylindrical cells. They rather frequently projected into the lumen of a duct, which was thus narrowed. In serial sections, it could be noted that these buds were occasionally small and consisted of but few cells.

There were occasions when the metaplasia of basal cells involved the whole circumference of the duct. Sometimes the terminal portions of the ducts could be seen filled by groups of cells arranged in a solid manner. One not infrequently got the impression that the proliferation of

TABLE 1.—*Conditions Associated with Metaplasia of Basal Cells in the Pancreas*

Case	Age	Sex	Disease	Accompaniment in Pancreas	Jaundice	Gallstones	Diabetes
1	24	M	Cardiac.....	Dilatation of ducts, enlargement of the islands of Langerhans, necrotic areas	+	0	0
2	19	F	Endocarditis.....	Small necrotic areas.....	0	0	0
3	70	F	Cholecystitis (stones); died after cholecystectomy	Lipomatosis.....	+	+	0
4	75	F	Carcinoma of the gallbladder	Liptomatosis, cysts, necrotic areas	+	+	0
5	56	F	Pulmonary embolus after colporrhaphy	Lipomatosis, cysts, adenoma of an island of Langerhans	0	0	0
6	27	M	Chronic endocarditis	Necrotic areas.....	+	0	0
7	46	F	Acute yellow atrophy of the liver	Necrotic areas.....	+	0	0
8	67	M	Carcinoma of the choledochus	Dilated ducts, increased connective tissue, slight lipomatosis	+	0	0
9	54	F	Cardiac insufficiency	Lipomatosis, enlarged islands of Langerhans	0	0	0
10	30	M	Periarteritis nodosa	Slight lipomatosis, dilatation of some ducts	0	0	0
11	19	M	Lymphogranulomatosis	Diffuse dilatation of ducts	+	0	0
12	47	F	Cholecystitis, cholelithiasis	Slight lipomatosis.....	+	+	0
13	62	M	Myocarditis.....	Lipomatosis, formation of cysts	+	+	0
14	36	F	Puerperal sepsis.....	.....	+	+	0

basal cells could bring about the formation of smaller or larger solid cell groups.

The metaplasia of basal cells was interpolated with the normal cylindrical epithelium, and could be observed in the various parts of the duct system. Even when the proliferation of basal cells was widespread, hornification was seldom observed. Masses of a horny substance arranged in concentric layers could, on a few occasions, be seen within the ducts. The proliferation of basal cells occurred most frequently in the tail of the pancreas.

The consequences of the metaplasia of basal cells in the ducts of the pancreas were observed to be, in reality, consequences of the narrowing or partial obliteration of the lumen of ducts.

Effects of Metaplasia of Basal Cells in the Ducts of the Pancreas on the Pancreas. 1. Flattening of the Cells of the Acini.—It is well known that the lumen of a pancreatic acinus, or end chamber, is normally hardly visible, because it is lined by cells of a high type. Dilatation of the lumen of an end chamber can be brought about by any factor which causes narrowing of a pancreatic duct and mechanical retention of pancreatic secretion. We believe that a metaplasia of basal cells in the duct of the pancreas can be responsible for such a change.

It depends on the location and extent of the metaplasia of basal cells whether the dilatation of pancreatic acini is general or circumscribed.

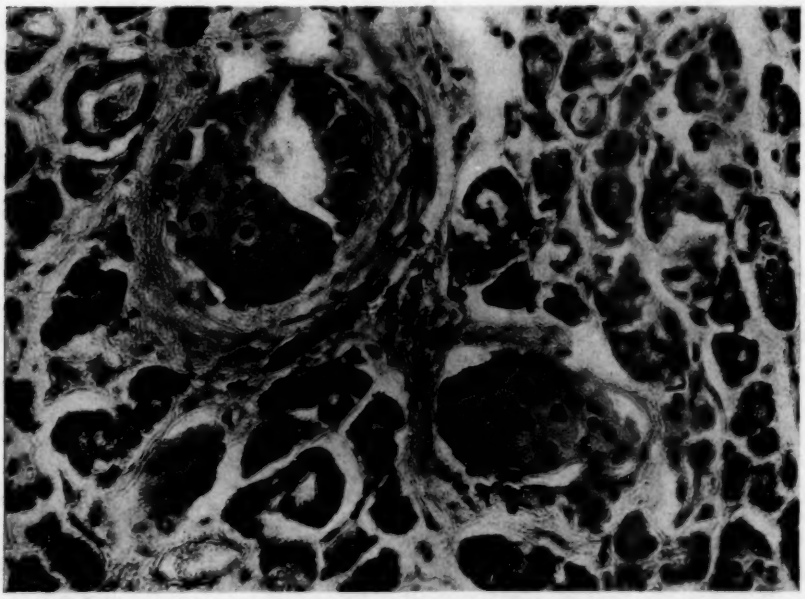

Fig. 1 (case 1).—High power magnification, showing metaplasia of basal cells in two small pancreatic ducts. The lumen of both ducts is greatly narrowed, but the metaplastic changes in each case occupy only a part of the duct circumference.

The dilatation of end chambers is such a common consequence of the metaplasia of basal cells that we used this phenomenon as a satisfactory indicator in collection of our cases. Thus, wherever we noted dilatation of end chambers we would always look for a metaplasia of basal cells in the corresponding ducts.

The cells which line the acinus in such cases are only as high as their nuclei, while the dilated lumen of the acinus can be filled by coagulated secretion. This flattening of the epithelium may in some instances be so uniform that were it not for the presence of the islands of Langerhans one could easily mistake such a section for one of thyroid tissue.

The extent to which the acini are dilated is variable and is naturally dependent on the degree to which the ducts have been narrowed.

2. Formation of Cysts.—In three of our cases a formation of cysts was found in the pancreas. The cysts varied in size from that of a millet seed to that of a pea. They were lined by cylindrical epithelium and in some places also by multilayer epithelium corresponding with the metaplasia of basal cells. Where cylindrical and stratified epithelium joined, the cylindrical epithelium sometimes covered the stratified epithelium in the same way as it does in erosions of the cervix uteri.

Priesel followed the relationship of the metaplasia of basal cells to the formation of cysts in the pancreas. In serial sections, he was able to

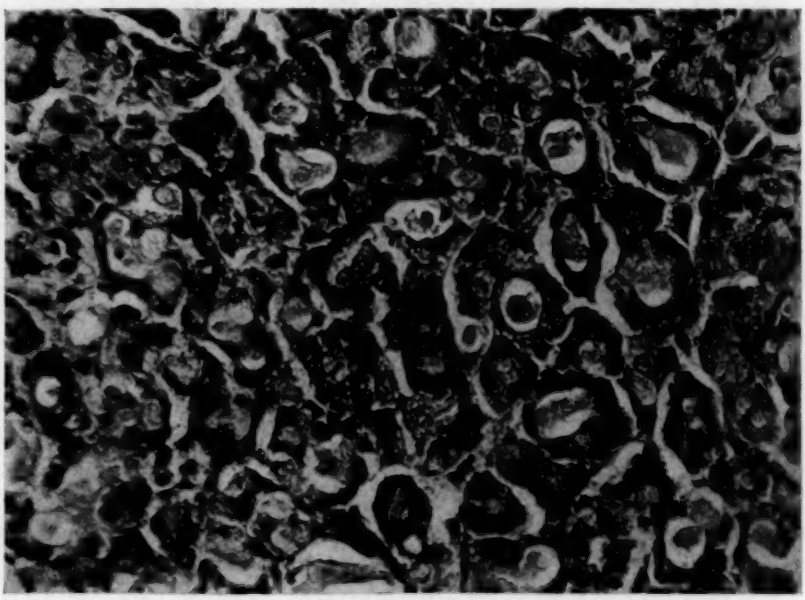

Fig. 2 (case 11).—Dilatation of end chambers due to the metaplasia of basal cells in small pancreatic ducts. The acini are filled by a homogeneous secretion, and the cells which line them are flattened.

prove that cysts are formed behind the metaplasia of basal cells in the ducts. We have confirmed these observations. We consider that the cysts are due to a dilatation of that portion of the secretory system which is behind the narrowing caused by the proliferation of basal cells. It is probable that proliferating basal cells, penetrating here from the proximal part of the ducts, can later on line these cysts.

Dilatation of the end chambers as a consequence of the metaplasia of basal cells in the ducts is a much more common occurrence than the formation of cysts. The dilatation of the end chambers on the one

hand or the formation of grossly visible cysts on the other likely depends on the localization of the metaplasia of the basal cells. In the latter case, the larger branches are evidently the seat of the metaplasia of basal cells.

3. Focal Necrosis.—Small necrotic areas the size of a poppy seed or larger were found in the pancreas in five of our cases (nos. 1, 2, 4, 6, 7). In three of these cases, endocarditis; in one, carcinoma of the gall bladder, and in the remaining one acute yellow atrophy of the liver were associated observations. Jaundice was present at necropsy in four of the cases.

Only those small necrotic areas around which some inflammatory reaction on the part of the surrounding tissue, but more especially leukocytic infiltration, could be noted were regarded as having occurred intra vitam. In another article [31] we pointed out that in cases in which jaundice due to alterations of the papilla of Vater occurs, retention of pancreatic secretion can also take place. We have already mentioned that in four of five cases of basal cell metaplasia which were complicated by focal necrosis jaundice was present. An ascending infection in the pancreas was therefore possible in these cases. The relationship between focal necrotic areas and the metaplasia of basal cells in the ducts could be twofold. The one might be that the metaplasia of basal cells plays a direct part in the production of focal necrosis, the other that both the focal necrosis and the metaplasia of basal cells are consequences of stasis or of ascending inflammatory processes in the pancreas. We shall see that not all cases of metaplasia of basal cells are to be interpreted as being due to stasis or to an ascending infection. It is probable that a metaplasia of basal cells acts rather as a factor predisposing the pancreas to focal necrosis; that when the retention of pancreatic secretion occurs, the consequences of such retention are more severe in those areas in which the metaplasia of the basal cells occurs and in which it has already narrowed the duct system.

4. Lipomatosis.—Another possible consequence of the metaplasia of basal cells in the pancreas is lipomatosis. We observed such changes in the pancreas in eight of the cases which we have reported. It was Priesel who first described the combination of lipomatosis, cyst formation and basal cell metaplasia in the pancreas. We have observed such a combination in our material on three occasions.

We believe that lipomatosis of the pancreas in the majority of cases results from the breaking down of pancreatic tissue, which is replaced by fat. Exact proof of the origin of pancreatic lipomatosis can be established only by quantitative chemical determinations and by the comparison of the fat content of the normal pancreas and of the pancreas

31. Baló and Ballon: Surg. Gynec. Obst.; to be published.

in which lipomatosis has occurred. One of us made these determinations and found that lipomatosis of the pancreas is mostly due to replacement of broken down glandular tissue by fat.

The breaking down of glandular tissue can take place in different ways. Focal necrotic areas were noted in five of our cases of metaplasia of basal cells. Such areas can later on be replaced by fat. Focal necrosis in the pancreas can recur, just as stasis of pancreatic secretion and an ascending inflammatory process can take place, repeatedly.

The sudden breaking down of pancreatic tissue takes place in the form of focal necrosis. The chronic retention of pancreatic juice is followed by a flattening of the cells lining the acini, a gradual disappearance of glandular tissue and by an increase in fat in the pancreas. This can be compared with the lipomatosis of the pancreas that results in animals following a ligation of ducts.

We believe that metaplasia of basal cells, by causing the narrowing of the lumen of ducts, can bring about alterations in the pancreas similar to those noted after experimental ligation of ducts.

5. Hypertrophy of the Islands of Langerhans.—The enlargement of the islands of Langerhans has been described by several workers. Cecil,[32] MacCallum,[33] Herxheimer [34] and many others long ago demonstrated the presence of degenerated and hypertrophied islands of Langerhans in the pancreas in cases of diabetes. That the islands of Langerhans enlarge as a result of obstruction or ligation of the pancreatic ducts is well known. Starvation may also cause them to enlarge.

Ssobolew [35] reported a case of diabetes in which an enlarged island of Langerhans had a diameter of 1.5 mm. He made mention of a "struma of the islands of Langerhans."

Since metaplasia of basal cells can also obstruct the lumen of ducts the question naturally arises as to what relationship metaplasia of basal cells bears to hypertrophy of the islands of Langerhans. For in several of our cases we found enlarged islands of Langerhans.

It has been proved that a regeneration of islands of Langerhans can take place from smaller ducts. There is thus the possibility that the buds which are formed in the early stages of a metaplasia of basal cells resemble a regeneration of the islands of Langerhans.

When the whole circumference of a duct is altered to epithelium of the multilayer type then this condition cannot be confused any more with a regeneration of the islands of Langerhans.

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32. Cecil: J. Exper. Med. **11**:266, 1909.

33. MacCallum: Am. J. Med. Sc. **43**:432, 1907.

34. Herxheimer: Virchows Arch. f. path. Anat. **183**:228, 1906.

35. Ssobolew: Virchows Arch. f. path. Anat. **177**:123, 1904.

It seems rather unlikely that the buds which we regard as expressions of a metaplasia of basal cells have anything to do with a regeneration of the islands of Langerhans.

We attempted to differentiate metaplasia of basal cells from regeneration of the islands of Langerhans histologically. Since Schultze [36] and Diamare [37] and subsequently Lane,[38] Bensley [39] and Bowie [40] were able to divide the cells of the islands of Langerhans into alpha, beta and gamma cells, we have a suitable means of making this differentiation.

Sections of proliferating duct epithelium showing a formation of buds which were suitable were stained by the Bensley stain. They failed to prove that these cells were similar to those of the islands of Langer-

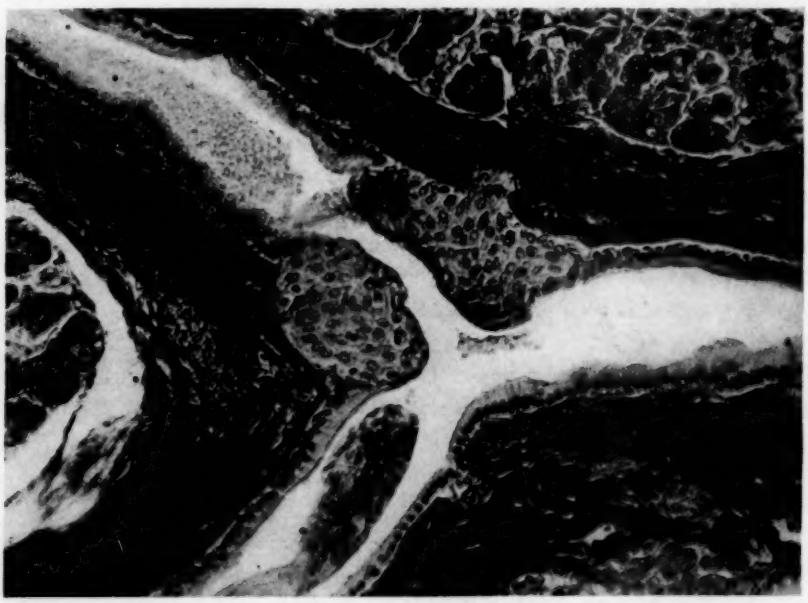

Fig. 3 (case 6).—Metaplasia of basal cells in a middle size pancreatic duct. It may be noted how the buds on opposite sides narrow the lumen of the duct.

hans. However, some of our sections were not suitable for staining by the Bensley stain. In addition to the absence of special granules, the lack of different cells and nuclei corresponding morphologically to the alpha, the beta and the gamma cells in the buds of the ducts speaks for a distinction between the metaplasia of basal cells of the ducts and a regeneration of the islands of Langerhans.

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36. Schultze: Arch. f. micr. Anat. **56**:491, 1900.
37. Diamare: Internat. Monatschr. f. Anat. u. Physiol. **16**:155, 1899.
38. Lane: Am. J. Anat. **7**:409, 1907-1908.
39. Bensley: Am. J. Anat. **12**:297 and 388, 1911-1912.
40. Bowie: Anat. Rec. **29**:57, 1924.

6. Adenoma of the Island of Langerhans.—In one of our cases we found an adenoma which took origin from an island of Langerhans. Such tumors have on different occasions been called, "adenoma of the island of Langerhans" (Heiberg [41]), "true adenoma of the pancreas" (Rollett [42]) and "adenoma arising from island cells" (Koch [43]), insulome (Masson [44]) and other such names. This is an observation comparatively rare, since but twenty cases have been reported.

The adenoma in our case measured 7 by 8 by 6 mm. It could be clearly seen with the naked eye. It was surrounded by a distinct capsule of connective tissue. The cells were arranged in rows, which were in reticular connection. Between the cells of the islands wide capillaries and blood sinuses were seen. The cells of the tumor were entirely similar to the cells of the islands of Langerhans elsewhere in the pancreas. Otherwise the pancreas presented a formation of cysts and lipomatosis. The cysts varied in size from that of a millet seed to that of a pea. The ducts leading to the area occupied by the adenoma were lined by multilayer epithelium. This epithelium sometimes blocked the ducts almost entirely, although in other instances smaller buds occurred. A direct connection between the proliferating epithelium of the ducts and the adenoma of the island of Langerhans could not be found. Enlarged islands of Langerhans were noted. They were sometimes embedded in fat tissue. Connective tissue septums were in many places thickened.

We would suggest that in the future only those enlargements of the islands of Langerhans that can be seen macroscopically should be placed in this group. Unless the term is reserved for such grossly visible tumors, it is impossible to make a distinct differentiation between an enlarged island of Langerhans that is a simple hypertrophy and a true adenoma. We do not believe that the presence of a capsule would help in this differentiation, because in cases of chronic pancreatitis the capsule cannot be differentiated from a proliferation of interstitial connective tissue.

The question may be raised as to the possible causes of such tumors. Besides the conditions already mentioned, it has been stated that the administration of pilocarpine arsenic, adrenalin, thyroid, phlorizine, rice and beef findings may cause hypertrophy of the islands of Langerhans. But it has recently been proved that some of these agents do not produce any such change.

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41. Heiberg: Centralbl. f. allg. Path. u. path. Anat. **22**:532, 1911.
42. Rollett: Frankfurt. Ztschr. f. Path. **10**:268, 1912.
43. Koch: Virchows Arch. f. path. Anat. **216**:25, 1914.
44. Masson: Les tumeurs, Paris, 1923, p. 442.

In a number of our cases the metaplasia of basal cells caused retention of pancreatic secretion, extreme dilatation of the pancreatic acini and flattening of their lining cells. We believe that a metaplasia of basal cells may produce the same alterations in the pancreas as is produced by a ligation of ducts, the only difference being that in the former case the ducts may be partially patent while in the latter they are entirely obliterated. A metaplasia of basal cells in the ducts may therefore cause a stasis of pancreatic secretion which is of longer duration than that which follows on ligation of ducts. After ligation of the pancreatic ducts the retention of pancreatic secretion lasts only for a short period,

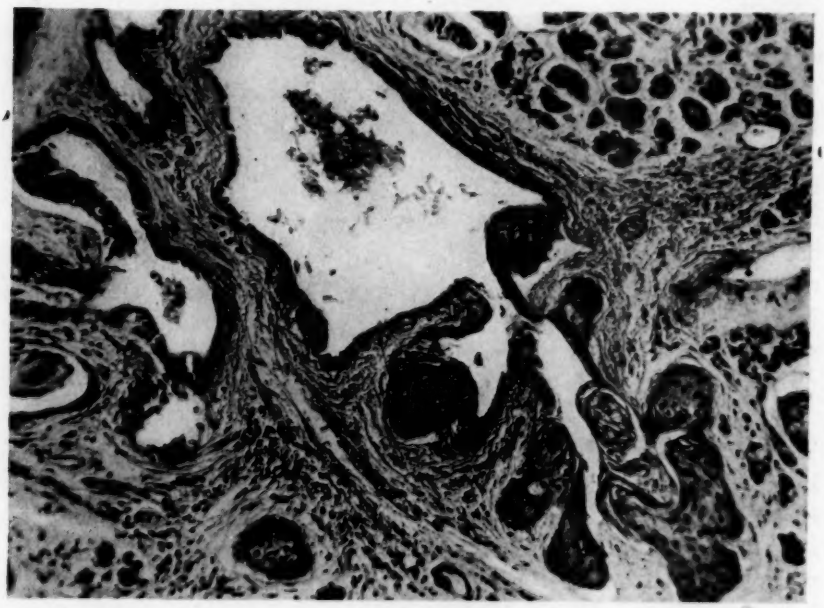

Fig. 4 (case 4).—Here may be noted how the proliferation of basal cells which fills the lumen of a small pancreatic duct presents characteristics that simulate invading growth.

because behind the point of ligation the cells of the acini stop secreting. If, however, the lumen of the ducts is only narrowed then the outflow of pancreatic juice is still possible and the cells of the acini will secrete for a longer period before they undergo atrophy. The irritation of the islands of Langerhans therefore continues over a longer period.

One other fact is perhaps worthy of mention. If the islands of Langerhans are to enlarge there must be sufficient room for them to do so. Thus, if there is already an increase of fibrous connective tissue around the islands of Langerhans, it is obvious that they cannot enlarge.

We believe that the breaking down or colliquation of acini produces conditions which are favorable for the enlargement of the islands of Langerhans.

In the twenty cases of adenoma of the island of Langerhans which we have collected from the literature, the metaplasia of basal cells in the ducts has been referred to only twice. But an accurate description of

TABLE 2.—*Adenoma of the Islands of Langerhans*

Case	Recorder	Age	Sex	Size of Tumor	Diabetes	Metaplasia of Basal Cells in Ducts	Jaundice	Gallstones
1.	Nicholls: J. Med. Res. **8**: 385 1902	..	..	3 by 2.5 mm.	..	..	..	..
2.	Helmholz: Bull. Johns Hopkins Hosp. **18**: 185, 1907....	65	M	5 by 3 by 5 mm.	0	..	..	..
3.	Morse: J. A. M. A. **51**: 1075 (Sept. 26) 1908.....	44	F	5 mm. in diameter	0	..	..	..
4.	Morse	46	M	3 mm. in diameter	0	..	..	..
5.	Cecil: J. Exper. Med. **13**: 595, 1911	63	M	4 by 3.5 mm.	0	..	..	..
6.	Heiberg: Centralbl. f. allg. Path. u. path. Anat. **22**: 532, 1911	64	F	6 by 5 mm.	+	..	..	..
7.	Alezais and Peyron: Compt. rend. Soc. de biol. **70**: 400, 1911	?	?	Of the size of a pea	..	..	..	..
8.	Rollett: Frankfurt. Ztschr. f. Path. **10**: 268, 1912.....	25	F	11 mm. in diameter	0	..	..	..
9.	LeComte: J. Med. Res. **29**: 251, 1913-1914	42	F	100 by 70 by 40 mm.	0	+	..	..
10.	Koch: Virchows Arch. f. path. Anat. **216**: 25, 1914.....	22	F	14 mm. in diameter	0	..	..	+
11.	Goldblatt: J. Cancer Res. **6**: 277, 1921	55	F	45 by 35 by 25 mm.	..	..	..	..
12.	Priesel: Frankfurt. Ztschr. f. Path. **26**: 453, 1922.....	63	F	12 by 10 by 9 mm.	0	..	..	..
13.	Priesel	51	M	9 by 10 mm.	..	+	..	0
14.	Priesel	60	M	3.5 by 2.5 by 2.5 cm.	0	..	..	..
15.	Schneider: Contribution a l'étude de l'adénome Langerhansien insulome, Thèse, Lausanne, 1924	84	M	15 by 10 mm.	0	..	..	..
16.	Schneider	70	F	50 by 45 by 30 mm.	0	..	..	..
17.	Lang: Virchows Arch. f. path. Anat. **257**: 235, 1925.....	39	F	5 mm. in diameter	..	..	..	..
18.	Warren: Am. J. Path. **11**: 335, 1926	53	F	1.7 by 1.7 by 1.4 mm.	0	..	..	..
19.	Warren	63	M	1.3 by 1.1 mm.	0	..	..	..
20.	Warren	49	F	1.2 by 1.2 mm.	0	..	..	..
21.	Warren	48	F	9 by 6.5 by 6 mm.	0	..	..	..
22.	Baló and Ballon: Surg. Gynec. Obst., to be published.....	56	F	7 by 8 by 6 mm.	0	+	0	0

the duct system is, in many instances, not given. We are not of the opinion that the metaplasia of basal cells is necessarily followed by the formation of an adenoma of the islands of Langerhans but rather that if the conditions present are those which we have just described, one of the consequences then may, in some instances, be the formation of an adenoma. Priesel endeavored to show that an adenoma of the islands of Langerhans may arise from the epithelium of the ducts. But only in his second case was he able to demonstrate any histologic connection between the duct and the adenoma of the island of Langerhans.

THE ETIOLOGY OF THE METAPLASIA OF BASAL CELLS IN THE DUCTS OF THE PANCREAS

In considering the etiology of the metaplasia of basal cells in the ducts of the pancreas, local and general factors must be dealt with. Proliferation of basal cells is a subject to which Krompecher gave much attention. Although most of his investigations were along morphologic lines, Krompecher nevertheless repeatedly turned to the question of the cause of benign and malignant proliferation of basal cells. He concluded that benign proliferation of basal cells is a preliminary to cancer, and is due to the action of various toxins, bacterial in origin. Of particular importance he thought was the local action of these bacterial toxins. One must not think that Krompecher was of the belief that these toxins alone were responsible for the metaplasia of basal cells. His writings show clearly that he was rather of the opinion that the problem of the metaplasia of basal cells, like that of carcinoma, would only be cleared up by considering a combination of several factors.

Recent experimental observations tend to prove that the metaplasia of basal cells is dependent not only on local conditions but also on the general status of the whole organism. Thus Wolbach and Howe [45] reported that the cylindrical epithelium of the nares, larynx, bronchi, submaxillary glands, prostate, epididymis and seminal vesicles of rats and guinea-pigs is transformed to epithelium of the keratinizing type if the animals are kept on a diet deficient in vitamin A. Goldblatt and Benischek [46] also recently reported that such a diet is adequate to induce metaplasia in the various types of epithelium. They state that this epithelial metaplasia is of such frequency in young rats so treated, as to constitute an acceptable morphologic indication of the deficiency of this vitamin in the diet. They claim that inadequate food, provided the vitamins are supplied, does not induce the metaplasia. We feel that metaplasia of basal cells as described by Krompecher and the transformation of cylindrical epithelium to epithelium of the stratified keratinizing type are but gradations of the same process. They do not essentially differ.

Wolbach and Howe emphasized the fact that the foci of keratinizing epithelium are of multiple origin. In some organs, they also observed a formation of cysts, which they considered had resulted from the retention of secretion through obstruction of the ducts by desquamated keratinized epithelial cells.

In our series of 14 cases, jaundice was found post mortem 6 times. In three instances, it was due to what is called "catarrhal jaundice," in

45. Wolbach and Howe: J. Exper. Med. **43**:753, 1925; Arch. Path. **5**:239 (Feb.) 1928.

46. Goldblatt and Benischek: J. Exper. Med. **46**:699, 1927.

one, to acute yellow atrophy of the liver, and in two, to the formation of a tumor. The catarrhal jaundice was of few days duration, while the jaundice in the cases of tumor extended for a much longer period. So, too, the metaplastic changes noted in the pancreas in cases of catarrhal jaundice were less extensive and less marked than when the jaundice had been of longer duration, as in the case of tumor. It was also to be noted that jaundice had been present at some time in the four cases of cholelithiasis, but this could not, as in the other six instances, be demonstrated at necropsy. This therefore makes a total of ten cases in which jaundice had at sometime been noted.

We observed metaplasia of basal cells in the pancreatic ducts in 8.75 per cent of all the cases which we examined. In five sevenths of these jaundice existed. The importance of jaundice is that simultaneously with it can occur the retention of pancreatic secretion from which an ascending inflammatory process in the pancreas can result. On this basis, retention of pancreatic juice, an ascending infection, or both, can be responsible for the metaplastic changes.

Can the retention of pancreatic secretion alone bring about metaplasia of basal cells in the ducts of the pancreas? A reply to this question can be given only by considering the consequences of a ligation of ducts. Kyrle and Weichselbaum found that the epithelium in the pancreatic ducts of animals proliferates shortly after the ducts have been ligated. They interpreted these changes as expressions of regeneration on the part of the islands of Langerhans.

Whether the proliferation of the epithelium in certain portions of the duct or under certain conditions leads to regeneration of the islands of Langerhans or to metaplasia of basal cells is a subject for further consideration. Suffice it to say that with special stains it is possible to differentiate the different cells of the islands of Langerhans from proliferating basal epithelium. The data available to date do not consider this point.

One must also decide whether or not an ascending inflammatory process takes place in the duct system of the pancreas after ligation of the duct. We conclude that the observations in experiments with animals do not speak against the possibility that metaplasia of basal cells in the ducts of the pancreas can result from the retention of pancreatic secretion plus an ascending infection. We have demonstrated signs of an ascending infection in our acute, as well as in our chronic cases. In the acute cases these were focal necrotic areas; in the chronic, lipomatosis. Lipomatosis is, in general, classified with chronic pancreatitis. Evidently the metaplasia of basal cells must be of a certain degree and duration to produce consequences in the pancreas. It is also true that not all ascending inflammations in the ducts of the pancreas bring about a metaplasia of basal cells. We examined several pancreases

in which signs of the former were evident but metaplasia of basal cells was absent.

In two sevenths of our cases, local causes, such as retention of pancreatic secretion or ascending inflammation, could not be demonstrated. We must therefore believe, as we have already noted, that not only local factors but also general ones may be responsible for basal cell changes in the pancreatic duct system. What these may be besides a deficiency in vitamin A we cannot tell.

It is interesting to note that metaplasia of basal cells in the ducts of the pancreas can occur also in younger persons. Other authors have stated that, in general, such changes in the ducts of the pancreas are usually noted in advanced age; according to Priesel, in most instances, in the seventh decade. In our own series it was noted in persons under 40 years, in six instances (42.85 per cent). It must of course be appreciated that such a figure will vary considerably if the necropsies from which material is drawn also include necropsies on children. Wilson and Du Bois [47] have reported metaplasia of basal cells in the ducts of the pancreas of a five months old child who had died from keratomalacia.

SUMMARY

Metaplasia of basal cells in the ducts of the pancreas was noted in 14 (8.75 per cent) of 160 pancreases, which were examined. Metaplasia of basal cells can occur in smaller or larger ducts, forming either circumscribed "buds" or an involvement of the epithelium of the ducts in its entire circumference. Varying directly with the size of the ducts involved, either dilatation of the end chambers or formation of cysts may result.

Metaplasia of basal cells in the duct system of the pancreas may be responsible for changes which are either acute or chronic. The acute are those which result from the retention of pancreatic secretion. They may be focal necroses. The chronic changes are similar to those which ensue after experimental ligation of the pancreatic duct. Lipomatosis of the pancreas may be one of them.

The blockage of pancreatic ducts by proliferative changes of the basal cells causes enlargement of the islands of Langerhans and in one instance which we report, an adenoma of an island. Proliferating basal cells in the ducts of the pancreas do not take the special stains used to differentiate the various cell types which make up an island of Langerhans.

In five sevenths of the cases which we report, jaundice had occurred at some period. This would suggest that in the majority of cases, either a retention of pancreatic secretion, an ascending inflammatory process or

47. Wilson and Du Bois: Am. J. Dis. Child. **26**:431 (Nov.) 1923.

both are present, and as local factors favor the basal cell changes. In two sevenths of our cases, local factors which would adequately explain the metaplasia of basal cells could not be found. It is possible that general factors similar to a deficiency of vitamin A may play some rôle in the proliferation of basal cells in the ducts of the pancreas.

Metaplasia of basal cells in our series occurred quite frequently in young persons.

MULTIPLE HEMANGIOMAS OF THE SKIN AND OF THE INTERNAL ORGANS*

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In the group of angiomatous tumors a form is sometimes referred to as offering great diagnostic difficulties because it behaves like a malignant tumor although the microscopic picture is not suggestive of it. These tumors have been used as an argument in favor of the conception expressed by some pathologists that there exists an essential similarity between innocent and malignant blastomas (Shennan,[1] Cathcart[2]). The signs of malignancy of the microscopically benign but potentially malignant angiomas are: their local invasive and destructive growth, their recurrence after surgical removal and their production of metastases to distant organs. There is, however, no uniformity of the histologic structure of these angiomas. Some of them grow by sprouting of capillary blood vessels, which later, becoming filled with blood, expand and cause a pressure atrophy of the invaded structures. They are simple cavernous hemangiomas with practically little, if any, stroma and a flat lining endothelium (Andrejew,[3] Berenbruch,[4] Hildebrand,[5] Konjetzny,[6] Schweizer,[7] Weiss,[8] Töpfer,[9] and others). There is a second group represented by a more complicated type, which shows a cellular or syncytial stroma sometimes described as embryologic. The blood vessels differentiate from this stroma, and in places may become so abundant as to obscure it (Borrmann,[10] Ewing,[11] von Falkowski,[12] Livingston and Klemperer,[13] Klinge,[14] Shennan,[1] Stamm[15]). In some

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1. Shennan T.: J. Path. & Bact. **19**:139, 1914.
2. Cathcart, quoted from Shennan (footnote 1).
3. Andrejew: Deutsche Ztschr. f. Chir. **201**:320, 1927.
4. Berenbruch, K.: Inaugural Dissertation, Tübingen, 1890.
5. Hildebrand: Deutsche Ztschr. f. Chir. **30**:91, 1890.
6. Konjetzny, G. E.: München. med. Wchnschr. **59**:241, 1912.
7. Schweizer, R.: Schweiz. med. Wchnschr. **4**: 243, 1923.
8. Weiss, S.: Med. Klin. **7**:1273, 1911.
9. Töpfer, D.: Frankfurt. Ztschr. f. Path. **36**:337, 1928.
10. Borrmann, R.: Ziegler's Beiträge **40**:373, 1907.
11. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1922.
12. Von Falkowski, A.: Ziegler's Beiträge **57**:385, 1914.
13. Livingston, S. F., and Klemperer, P.: Arch. Path. & Lab. Med. **1**:899 (June) 1926.
14. Klinge, F.: Deutsche Ztschr. f. Chir. **183**:195, 1923.
15. Stamm, R.: Inaugural Dissertation, Göttingen, 1891.

instances, a marked hyperplasia and proliferation of the endothelium have been observed (Shennan,[1] Stamm[15]) with a differentiation of the large endothelial cells into blood cells (von Falkowski,[12] Klinge[14]). One fully agrees with Klemperer that in the presence of such microscopic observations one should not speak of a benign tumor, although anaplasia of the cells is not present. These cases prove that malignancy cannot be considered simply from the standpoint of the cellular structure. There are, also, transitional stages between these forms of hemangiomas and sarcomatous angiomas, which are vascular tumors with a sarcomatous stroma (Jores,[16] Langhans,[17] Theile,[18] Ramdohr[19] and others).

Association with what were supposed to be metastases was observed in both groups of hemangiomas, in the simple cavernous (Konjetzny,[6] Weiss[8]) as well as in those with embryonic stroma (Borrmann,[10] Klinge,[14] Shennan,[1] Stamm[15]). In the cases described by Livingston and Klemperer[13] and Ewing,[11] postmortem examinations had not been made, but in the latter case an involvement of the lungs was suggested by the clinical observations. At least as far as the hemangiomas with a mature stroma are concerned, their possible formation of metastases is doubtful. Multiplicity of hemangiomas of the skin and mucous membranes is common, and is explained by the fact that the hemangioma is a congenital formation the origin of which can be traced to the first anlage of the vascular system in early embryonic life (Ribbert[20]). The occasional appearance of these tumors in later life, as in familial epistaxis (Goldstein,[21] Osler,[22] Steiner,[23] Williams,[24]), does not speak against their congenital character, which is also evident from the heredity. Hemangiomas of the skin not seldom occur with similar tumors of the liver (Brüchanow,[25] Roggenbauer,[26] Fischer[27]). An interesting condition, described as Lindau's disease,[28] presents cystic angiomas of the cerebellum associated with an angiomatosis of the retina (von Hippel's disease), angiomas of the spinal cord, cystic formations

16. Jores, L.: Centralbl. f. allg. Path. u. path. Anat. **19**:662, 1908.
17. Langhans, T.: Virchow's Arch. f. path. Anat. **75**: 273, 1879.
18. Theile: Virchow's Arch. f. path. Anat. **178**:296, 1904.
19. Ramdohr, M.: Virchow's Arch. f. path. Anat. **73**:459, 1878.
20. Ribbert, H.: Geschwulstlehre Cohen, 1911.
21. Goldstein, H. I.: Arch. Int. Med. **27**:102 (Jan.) 1921.
22. Osler, W.: Bull. Johns Hopkins Hosp. **12**:333, 1901.
23. Steiner,: Arch. Int. Med. **19**:194 (Feb.) 1917.
24. Williams, C. M.: Arch. Dermat. & Syph. **14**:1, 1926.
25. Brüchanow: Ztschr. f. Heilk. **20**:1, 1899.
26. Roggenbauer: Ziegler's Beiträge **49**:313, 1910.
27. Fischer, B.: Pathologie des Angioms, Ergebr. d. allg. pathol. u. path. Anat. **10**:815, 1904-1905.
28. Lindau, A.: Acta path. et microbiol. Scadinav., 1926, suppl. 1.

of the pancreas and of the kidneys and hypernephromas (Rochat,[29] Schuback [30]).

Finally, there are the cases of numerous hemangiomas in different organs, including such unusual sites as the lungs, the heart, the thyroid and the uterus. From the literature, I have collected the following combinations of sites: Skin, liver, spleen and bones of the skull (Billroth [31]); in a case of Lindau's disease, involvement of the base of the skull, the bone marrow and the urinary bladder (Brandt [32]); skin, liver, parotid gland and intestine (Gascoyen [33]); skin, mucous membranes, mesentery and retropleural tissue (Ghon [34]); skin and pia mater (Kalischer [35]); liver and bones of the skull (Major and Black [36]); liver, suprarenal gland, ovaries and uterus (Payne [37]); skin, epicardium, pleura, peritoneum and choroid plexus (von Rokitansky [38]); skin, mucous membranes, liver and heart (Runge [39]); cerebellum, medulla oblongata, spinal cord, liver and urinary bladder (Tannenberg [40]); (1) skin, mucous membranes, submaxillary gland, thyroid, liver, lung, intestine and urinary bladder and (2) skin, mucous membranes, mediastinum, pericardium, liver and left kidney (Töpfer [41]); skin, mucous membranes, peritoneum and liver (Ullmann [42]), and liver and bones of the skull (Virchow [43]).

To these cases, I wish to add a new one which is of interest especially with regard to the question of metastasizing.

REPORT OF A CASE

History.—A white girl, aged 4 days, was brought to the Cook County Hospital by her father, who said that at birth a marked swelling of her neck had been noted. The family history and the details of the birth of the child were not significant.

The clinical examination showed a female infant 44 cm. long and 2.6 Kg. in weight. On the left side of the neck was a large, purplish gray tumor-like mass,

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29. Rochat, G. F.: Klin. Monatsbl. f. Augenh. **78**:601, 1927.
30. Schuback, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **110**:359, 1927.
31. Billroth, T., quoted from Veeder, B. S., and Austin, I. H.: Am. J. Med. Sc. **143**:102, 1912.
32. Brandt, R.: Graefe's Arch. f. Ophth. **106**:127, 1921.
33. Gascoyen: Tr. Path. Soc. London **2**:267, 1860.
34. Ghon, A.: Verhandl. d. Deutsch. Path. Gesellsch. **12**:242, 1908.
35. Kalischer, S.: Arch. f. Psychiat. **34**:171, 1901.
36. Major, R., and Black, D. R.: Am. J. Med. Sc. **156**:469, 1918.
37. Payne: Tr. Path. Soc. London **20**:203, 1869.
38. Von Rokitansky, C.: Manual of Pathological Anatomy, Blanchard and Lea, Philadelphia, 1855.
39. Runge, H.: Arch. f. Gynäk. **122**:491, 1924.
40. Tannenberg, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **92**:119, 1924.
41. Töpfer, D.: Frankurt. Ztschr. f. Path. **36**:337, 1928.
42. Ullmann, K.: Sitzungsb. d. Ges. d. Ärzte Wien, March 10, 1899.
43. Virchow, R.: Die krankhaften Geschwülste, Berlin, 1863.

which reached from the anterior median line to the posterior margin of the left sternocleidomastoid muscle and from the shoulder joint to the mastoid region, pushing up the left ear. The tumor was sacculated, soft and compressible. The skin was not adherent; there were not any thrills or pulsations. From the tumor, pure blood was aspirated which gave a negative Wassermann reaction.

Scattered over the body in the skin were about 100 small nodules varying in size from that of a pinpoint to that of a diameter of 1 cm. These were purple, sometimes with a gray tint. The nodules were elevated over the skin and were firm.

The child had a persistent discharge from the vagina, in which on several occasions gram-negative diplococci resembling gonococci were found. After the child had been in the hospital eight months, it contracted measles, from which it recovered. During the entire period of observation the tumor in the neck remained unchanged.

The patient was discharged from the hospital, but was brought back a month later acutely ill with fever, restless, and refusing food. A diagnosis of lobular pneumonia of the right upper lobe was made. The tumor of the neck had greatly decreased in size. The overlying skin was wrinkled, and a baggy mass was felt under it. The purple nodules of the skin still were present.

The infant was transferred to the Research and Educational Hospital of the University of Illinois. It grew weaker rapidly and developed an acute suppurative condition of the right thigh. The temperature went up to 106 F. A spinal puncture made immediately before death showed a turbid fluid under increased pressure, which gave a positive reaction in the Ross-Jones test and which contained 800 leukocytes per cubic millimeter. Cultures made from this fluid yielded hemolytic streptococci.

Autopsy.—The body was that of a poorly nourished white girl, 68 cm. long and weighing 4,800 Gm. The skin was studded with sharply demarcated, dark violet, round patches, varying from 1 to 4 mm. in diameter. These patches were most numerous on the right side of the face, around and inside the right ear, on both sides of the chest and on the left side of the back. Though scanty on the extremities, they were also found on the soles of the feet and on the palms of the hands. In the right groin, a fluctuating swelling was present. The skin over the left side of the neck appeared wrinkled, and a dense mass was felt underneath it. This mass contained several hard nodules.

On removal of the soft parts of the head, a cavity was discovered between the galea aponeurotica and the lower posterior angle of the right parietal bone. The cavity contained thick, dark yellow pus, which extended through an irregular penetrating defect in the bone to the external surface of the dura mater. The internal surface of the dura was smooth and shiny.

At the base of the brain, much thick yellow pus had accumulated. Pus covered also the convex surface of the hemispheres, being most abundant near the medial margin and about the poles of the temporal lobes. In the region of both sylvian fissures, a deep blue mass shone through the opaque leptomeninges. The mass covered the lower third of the posterior central convolution and extended backward to the interparietal sulcus. When these areas were cut through, there was revealed the presence of dilated and convoluted, thin walled blood vessels, which were from 1 to 2 mm. thick. The brain was soft; the ventricles were dilated and filled with much cloudy fluid.

The accumulation of pus in the subarachnoidal spaces continued into the spinal canal. The inside of the dura mater showed, in its ventral aspect, at the height of the upper cervical region, a number of flat, deep blue elevations, filled with blood. The anterior roots of the second and third cervical nerves were thicker than they normally are and grayish red.

Dissection of the left side of the neck exposed a firm fibrous mass, covered by but not fixed to the skin, extending from the clavicle to 3 cm. below the mastoid bone. This mass was adherent to the superficial fascia. It contained several calcified nodules.

The thyroid weighed 2.5 Gm. It was grayish brown and firm. It contained a number of deep red, round areas measuring from 0.5 to 1.5 mm. in diameter.

The thymus was made up of firm, grayish white tissue, and weighed 2 Gm.

The lungs were free. The surface appeared a light grayish red. The interlobular septums were distinct as white lines. Scattered over both lungs were numerous deep red, slightly elevated, round areas from 0.5 to 3 mm. in diameter (fig. 1). They were located in the interlobular septums. About fifty of these patches were counted on the surface of each lung. The lungs were filled with air except for some irregular areas along the anterior margin of both the upper and the lower lobes, where the tissue was firm and deep grayish red.

Fig. 1.—The left lung, showing multiple subpleural hemangiomas.

The heart weighed 35 Gm. and was of normal shape. Changes were not observable in the valves; the intima of the aorta was smooth. The small intestine, on the surface, showed many irregularly shaped, dark red patches, varying in diameter from 2 to 6 mm., which were located opposite the mesenteric attachment (fig. 2). The patches were composed of tortuous tufts located in the wall and shining through the serosa and mucosa. The sigmoid, near the anterior margin of the mesocolic attachment, contained a mass of convoluted vessels. This mass was 18 mm. long, 15 mm. wide and from 5 to 7 mm. thick. It contained a few small, stony concretions.

The liver weighed 238 Gm., the spleen 18 and the pancreas 7. These organs did not reveal anything unusual. The same can be said about the suprarenal gland, the kidneys, the stomach and the sex organs.

In the left groin, an extensive suppuration extended between the adductoric muscles down into the region of the median epicondyle.

The bone marrow in the middle of the right femur was soft and reddish brown. Smears made from the pus between the leptomeninges and in the groin showed numerous long chains of streptococci.

Microscopic Examination.—The violet patches of the skin were wide, blood-filled spaces located in the subepidermal part of the cutis. They were lined by a flat endothelium and were derived from capillary vessels, which opened into them abruptly. Similar, smaller blood-filled spaces were present in the deeper layers of the cutis, where they often arranged themselves about the sweat glands.

The convoluted masses of the leptomeninges, which filled the sylvian fissures, were composed of wide blood vessels. The walls of these vessels were formed by from two to three layers of fibrillar connective tissue lined by a flat endothelium (fig. 3). The vessels were filled with blood, which in places had coagulated. From them originated narrow branches which entered the cortex of the brain. The blood vessels in the brain did not show abnormal widening. Between the leptomeninges there was much exudation consisting of a network of fibrin and polymorphonuclear and mononuclear cells.

Fig. 2.—The small intestine, showing multiple hemangiomas.

Transverse sections through the right parietal bone showed a filling of the spaces of the diploë by loose, fibrillar connective tissue, in which were embedded numerous wide and thin-walled blood vessels. Here and there a small group of myelocytes was found. The bone appeared normal.

The body of the third cervical vertebra contained active bone marrow with small foci of degeneration and an intracellular accumulation of fibrinous exudate. Abnormal widening of the blood vessels was not seen.

In the ventral portion of the upper cervical dura mater were numerous round and oval blood-filled spaces. They were located near the internal surface and protruded somewhat into the spinal canal.

In the spinal cord a diffuse capillary hyperemia and degenerative changes of the ganglion cells of the anterior horns were observed. The blood vessels appeared normal. The anterior roots of the upper cervical nerves, described macroscopically as thickened and reddish gray, showed a striking predominance of the blood vessels (fig. 4). The vessels divided dichotomically, ran parallel with the nerve fibers, from which they were separated only by a flat endothelium, and occupied almost one fourth of the transverse diameter of the nerve.

In the thyroid, the interlobular septums were thicker than normal and contained many wide blood vessels of various shapes (fig. 5). These vessels extended into the lobules and came in close contact with the follicles, which showed signs of compression. The shape of the vessels depended on their size; that is, the smaller vessels were of irregular shape with lakelike outlines and gave off branches which

Fig. 3.—Hemangioma of the leptomeninges in the region of the sylvian fissure. Between the leptomeninges there is purulent exudation. Magnification, 15 times.

Fig. 4.—Hemangioma of the left anterior root of the third cervical nerve. Magnification, 25 times.

originated from them with a funnel shaped widening. The larger spaces were round or oval and were connected only with an afferent and an efferent vessel. The largest spaces often contained coagulated blood with beginning organization of the thrombus. All the spaces had a lining of flat endothelium.

The pleura of the lung was composed of several layers of fibrillar connective tissue. Where the interlobular septums were given off, round or oval blood-filled

spaces were present, which often bulged into the adjacent alveolar spaces. The alveoli bordering the septums had thick walls and were narrow, while near the center of the lobules the alveoli were wide. The dense areas near the anterior margins contained cellular exudate.

Fig. 5.—Hemangioma of the thyroid. Compression of the follicles is shown. Magnification, 120 times.

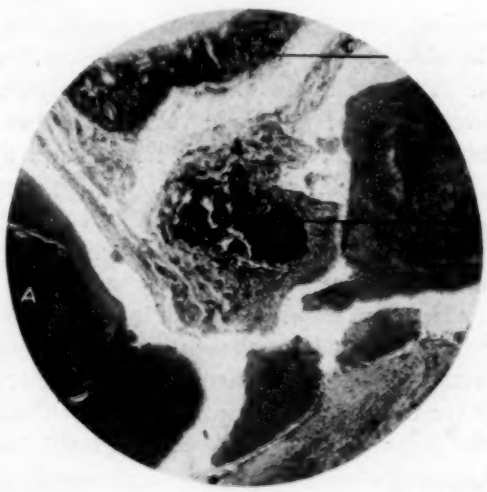

Fig. 6.—Hemangioma of the sigmoid. *A* indicates spaces filled with blood in the submucosa and the muscularis; *B*, a calcified thrombus, and *C*, mucosa. Magnification, 60 times.

The submucosa of the small intestine was distinctly thicker than normal and contained wide blood-filled spaces which compressed the muscularis. The spaces showed baylike extensions, which led into capillary blood vessels that ran towards the mucosa. In some of the spaces, recently formed thrombi were attached to the

lining of flat endothelium. The distribution of the vessels was best studied on small pieces of the intestinal wall, cleared and mounted in toto. In these preparations, straight and narrow vessels were seen running parallel to the surface. They widened suddenly and formed coils, from the convexity of which the smaller branches originated. Single coils were much distended and appeared as blood-filled lakes.

The abundance of the blood vessels was most marked in the portion of the sigmoid described as a convoluted, dark violet mass. The intestinal wall here consisted chiefly of blood vessels, which also occupied the muscularis and produced nodular projections on the serosa. The mucosa was thin and atrophic. Many of the wide vessels contained blood clots. These clots underwent calcification and led to the formation of stony concretions embedded with dense connective tissue (fig. 6).

In the spleen, a few round or oval blood-filled spaces were found about the follicles. The latter were small and often contained a center composed of epithelioid cells with granules of hemosiderin.

Abnormal blood vessels were not found in the kidneys, suprarenal glands, ovaries, and pancreas.

Anatomic Diagnosis.—Purulent streptococcal meningitis; subcutaneous abscess in the right posterior parietal region with erosion of the parietal bone and external pachymeningitis; deep phlegmon of the left thigh; confluent bronchopneumonia in both lungs; accidental involution of the thymus; fatty infiltration of the liver; cloudy swelling of the kidneys, and multiple hemangiomas of the skin, the leptomeninges, the anterior roots of the cervical nerves, dura mater spinalis, the diploë of the parietal bone, the thyroid, the lungs, the intestine and the spleen.

COMMENT

Studying the microscopic sections of this case, one obtains the impression that in many organs there was a marked disproportion between the development of the blood vessels and that of the specific structures. The blood vessels seemed to have grown much the faster and this excessive growth affected both their length and their circumference. They became too long and too wide, forming tortuous coils and lakelike spaces of blood. The tortuous portions gave off branches, while the wide spaces were connected only with an afferent and an efferent capillary blood vessel. Endothelial hyperplasia was not present.

In the introduction it was mentioned that in some hemangiomas the newly formed vessels differentiate from an indifferent cystogenic mesenchyma. I have recently seen two such tumors in animals. One was a mixed hemangio-lymphangioma which had replaced large portions of the liver of a dog and had also involved the ligamentum hepatoduodenale and a group of lymph glands at the hilum of the liver. The second tumor was one in a cow, shown to me by Dr. E. Day. In both lungs of the cow, there were multiple angiomatous tumors composed of a markedly cellular stroma, from which blood vessels developed through blood cells accumulating between the spindle-shaped cells and pushing them apart. Such stroma was not present in the multiple hemangiomas

of the child, although in some organs, especially in the lungs, the intestine and the thyroid, the abnormal growth of the blood vessels was associated with a marked increase of the surrounding connective tissue. This connective tissue, however, was mature, without any blood vessel and blood cell forming potencies, as in the cases reported by von Falkowski,[12] Klinge [14] and others. The angiomas arose from the preexistent capillary blood vessels by a giant growth of more or less circumscribed segments.

One may ask whether a lesion of this character should be classified as a hemangioma, a term which means a true vascular tumor. It is difficult to draw a sharp borderline between angiomas and angiectases, but in the presence of signs of an active growth, as compression and involvement of the surrounding structures, the determination of a vascular formation as tumor cannot be objected to. This tumor is a hamartoma with a tendency to continuous growth, although this tendency may be insignificant.

Some of the hemangiomas showed signs of spontaneous regression. There was a thrombosis of the wide blood-filled spaces with organization and calcification, which terminated in the complete obliteration of these spaces. The child had been suffering from repeated severe infections; it is known that intercurrent diseases may initiate the regression of hemangiomas.

Many of the patients afflicted with multiple hemangiomas die in early childhood, whatever the microscopic picture of the tumors may be (Brüchanow,[25] von Falkowski,[12] Kalischer,[35] Konjetzny,[6] Ramdohr,[19] Runge,[39] Stamm,[15] Töpfer,[9] Weiss [8]). If the patients attain a later age, some of the tumors may have reached a considerable size, since they keep on growing. There is a difference in the rate of growth, some of the multiple tumors growing faster than others. Even at the time of birth, there is a marked difference in the size and the extension of the angiomas. Thus, in my case, the swelling of the neck was first the outstanding feature, which later decreased considerably, but at the autopsy table the most pronounced vascular changes were found in the leptomeninges and in the sigmoid. In the spleen, they were visible only under the microscope. The predominance of the vascular new growth in one region of the body does not indicate that this new growth is the primary tumor and that the smaller formations scattered over the other organs are metastases. It is much more likely that all the tumors arose at the same time independently of each other. It is not necessary to assume that blood vessel formation elements are transplanted from a primary focus by way of the blood or lymph stream to a distant organ. Indeed this implantation has never been demonstrated even in the cases of hemangiomas with an indifferent stroma and hyperplastic endothelium.

The multiple cellular hemangiomas in the lung of a cow, which were mentioned, could be traced to small islands of a cellular mesenchyma which were found in the corners of the alveolar septums and around the smaller bronchi and which did not show evidences of a metastatic origin.

SUMMARY

A case of multiple hemangiomas of the skin and the internal organs in an infant is described. There was a marked difference in the size of these tumors, which in some organs showed signs of expansive growth. In spite of these observations, all the tumors were considered to be simultaneous and independent formations. The existence of a so-called benign, metastasizing hemangioma is doubtful.

DIFFUSE, OBLITERATING ENDARTERITIS OF UNKNOWN ETIOLOGY

AN INSTANCE WITH OBLITERATION OF THE INFERIOR VENA CAVA *

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This communication concerns an unusual instance of a diffuse, obliterating proliferation of the intima of the medium-sized and smaller arteries of the brain, heart, lungs, spleen, kidneys, thyroid and extremities associated with complete occlusion of the inferior vena cava. This widespread obliterative process resulted in profound anatomic changes in the viscera involved, with concomitant protean clinical manifestations. The process in all the vessels was limited almost in its entirety to the intima. We are unable to establish the exact nature of the process.

Bronson [1] reported an instance of diffuse obliterative arteritis of the vessels of the extremities, kidneys and brain in a boy aged 12. The patient did not show evidence of syphilis. The right kidney was irregularly contracted; the left was larger than normal but did not show any other change. There was a widespread obliteration of the small arteries and arterioles due to proliferation of the intima only, with atrophic changes in the parenchyma.

A thorough search of the literature did not reveal another similar instance.

REPORT OF A CASE

History.—A woman, aged 47, entered the Montefiore Hospital on Dec. 11, 1927, complaining that the left side of the body had been weak for one year and that she had been unable to read, write or understand spoken language for three years. She had been married thirty-two years and had had two children, the younger aged 29. After the birth of her second child, she had had five miscarriages. Five years before admission she had developed a varicose ulcer, which had healed slowly. About the same time she became increasingly irritable and one year later suddenly lost the ability to write. Subsequently, her understanding of spoken and written words rapidly disappeared. About this time, she complained of compression pains over the precordia, which were severely aggravated by slight exertion. Two years later she had a "stroke" and developed a complete left sided hemiplegia with diplopia, aphasia and inability to swallow properly. Her condition improved in the course of the next three weeks. However, she lost interest in her friends and became emotionally unstable. She continued to complain of precordial pain

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1. Bronson, W. P. S.: Obliterative Arteritis, Tr. Path. Soc. London **55**:212, 1905.

on slight exertion. During the few weeks prior to her admission, she had several peculiar attacks consisting of rigidity of the muscles of her trunk and extremities, accompanied by snorting respiration. During one of these attacks the patient had a chill and transient dimness of vision, and lost her ability to recognize objects or their use.

Physical Examination.—The patient was moderately obese and emotionally unstable. The superficial veins of the abdomen, groins and upper half of the thighs were markedly dilated and tortuous. A slight cyanosis of the lips was present. The left pupil was larger than the right, though both reacted to light and accommodation. The sclera had a subicteric tint. The heart was enlarged to the left; murmurs and other irregularities were not noted. The pulses were equal and regular; the blood pressure was 110 over 80. The lungs were normal. Healed ulcerations were present on both legs.

There was a mixed aphasia, also apraxia, left-sided astereognosis, emotional instability and residual signs of left hemiparesis with a questionable pseudobulbar speech.

The urine was normal. There was a slight secondary anemia. The Wassermann test of the blood was four plus; of the spinal fluid, negative. An x-ray photograph of the heart showed enlargement of all the chambers. The results of the chemical examination of the blood were normal.

The clinical diagnosis was cerebrospinal vascular syphilis.

Course.—The patient left the institution unimproved. She continued to complain of shortness of breath. On one occasion she expectorated clots of dark reddish-brown blood. On a return visit for antisyphilitic treatment, she suddenly developed a right hemiplegia. A few days later the temperature rose to 101 F. The pulse and respirations were rapid. She became drowsy. Coarse, moist râles were heard over both bases of the lungs. She died with pulmonary edema on Jan. 19, 1928.

Autopsy.—Examination of the body ten hours after death showed generalized endarteritis (possibly syphilitic); occlusion of the descending branch of the left coronary artery; healed infarct of the left ventricular wall; multiple infarcts of the brain; organized thrombosis, with complete occlusion of the inferior vena cava from the level of the renal veins to the femoral veins; healed infarct of the lung; healed infarct of the spleen; ulcers of the leg; multiple adenoma of the thyroid; primary contracted kidneys (arteriolosclerosis), and acute purulent leptomeningitis.

On the external surfaces of both legs were several small ulcerated areas. The large toes of both feet were deep bluish black. The lower abdominal veins were prominent and tortuous.

The heart weighed 400 Gm. All the chambers were dilated, and the muscle was hypertrophied. The myocardium showed numerous fibrotic streaks. In the right ventricle, a large, lamellated, irregular, yellowish-white mass, 6 cm. by 3 cm., was closely adherent to the anterior surface of the lower half of the ventricular wall. Beneath this, the heart muscle was thinned down to about 3 mm. and was densely scarred. The layers of the pericardium over this area were adherent. The interventricular branch of the left coronary vessel, for a distance of 3 cm. from its opening, was completely obliterated by a yellowish-white, firmly adherent mass.

The right kidney weighed 100 Gm., the left 80 Gm. The capsules stripped with slight difficulty. Both kidneys were firm, deep red, and the surface was coarsely granular. On section, the cortex was found to be narrow; the glomeruli were indistinct.

A few linear streakings and irregular yellowish plaques were present in the intima of the ascending portion of the aorta and in the abdominal aorta just distal to the mesenteric vessels. The inferior vena cava, from its origin to just below the entrance of the renal veins, was completely occluded by a firm, grayish-white, adherent mass which tapered for a distance of several centimeters above the point of complete occlusion. The vein in its greater portion was a fibrous cord. The common iliac veins were involved in the process, and the left renal vein was partly occluded by organized mural masses.

Several well defined nodules were found in the thyroid gland. On section, these appeared yellowish-brown; some were cystic.

The other organs did not show abnormalities.

Fig. 1.—A section of coronary vessels, magnified 10 times, showing marked proliferation of the intima of several branches with almost complete obliteration of the lumen.

The meninges over both convexities of the brain were infiltrated with greenish pus, which was apparently confined to the subarachnoid space. Both occipital regions were atrophied with softening in the right and left parietal regions. The blood vessels did not show any arteriosclerotic changes. In both hemispheres, there was softening extending from approximately the sylvian fissure back to the occipital pole. Ventrally, this softening extended into the upper thirds of the right and the left putamen and of the right thalamus. The capsule was more involved on the right than on the left. The ventral half of the neostriatum and of the thalamus were grossly intact, as well as the corpus callosum. A cyst 0.5 cm. in diameter adjoined the left calcarine fissure. In horizontal sections through the corpus callosum and the upper third of the caudate nucleus, appeared two areas of organized softening in the left hemisphere, one destroying the posterior third of the left frontal lobe and extending far into the corona radiata, and the other in the posterior half of the parietal region mainly confined to the cortical layers with

smaller foci irregularly distributed in the cortex of the right central parietal and occipital regions. In the section through the striatum, pallidum and thalamus, and the upper third of the cerebellar vermis, the whole parietal lobe was destroyed in the left hemisphere.

The spinal cord was grossly normal.

Microscopic Examination.—In the heart, at the site of the infarct, the muscle was thinned out and practically replaced by fibrous connective tissue. Beneath this, the muscle fibers contained a considerable amount of fat; the nuclei were pyknotic. On the endothelial surface of the infarct, there was an organized thrombus fused with the underlying fibrous plaque. Other sections of the myocardium showed fibrotic streaks and small foci of round cells in the interstitial tissue. An arteriole was obliterated by a mass of fibrous tissue which contained a small space lined

Fig. 2.—A section of the lung, magnified 40 times, showing complete obliteration of the lumen of a small artery with recanalization. Signs of acute bronchitis appear close to the vessel.

with endothelium. The coronary arteries showed extensive obliteration by masses of connective tissue, which extended directly from the intima and were continuous with it. There were spaces lined with endothelium in these occluding masses. The connective tissue contained a few cellular elements. The elastic coat was intact, the entire process being limited to the intima.

The bronchi were filled with pus; the mucosa was in part desquamated. There were small abscesses in the parenchyma of the lungs; edema and congestion were present. Several of the pulmonary vessels were partly occluded by thickening of the intima. A few small arteries were filled with a mass of connective tissue containing several large spaces lined with endothelium. The smaller spaces were not surrounded by a layer of elastic tissue.

In the liver, there was slight cloudy swelling, but changes were not observable in the vessels in the sections examined.

In the spleen, congestion and a scant cellularity of the pulp were observed. The malpighian corpuscles were small. The arterioles in the corpuscles and the small arteries showed a striking proliferation of the intima, which in many instances completely filled the lumen. The elastic layer was somewhat thickened in some of the vessels. Several small, healed infarcted areas were seen and one recent hemorrhagic infarct.

The cortex of the kidneys was markedly narrowed. The arterioles and the small and medium sized arteries showed extensive changes, particularly of the intima. The lumen of some of the vessels was narrowed to a mere slit; in others it was completely occluded by a mass of connective tissue in direct continuity with the intima, sparsely cellular, and containing one or two spaces lined with endothelium. Pigment was not present in the mass. The elastic coat of the arteries

Fig. 3.—A section of the kidney, magnified 20 times, showing a mass of connective tissue extending from the intima and filling the lumen of a small artery. Blood spaces lined with endothelium may be seen in the occluding mass.

was intact and, in places, strikingly thickened. The glomeruli were, in places, completely replaced by concentrically arranged lamellae of connective tissue. All graduations were seen, from moderate thickening of Bowman's capsule to complete replacement of the tuft by connective tissue. The accompanying tubules were atrophic; some contained a homogeneous material that stained pink. The interstitial connective tissue was increased and infiltrated with round cells. The areas of involvement occurred at more or less regular intervals in the cortex, the intervening tissue appearing normal.

There was little lipoid material in the cortex of the suprarenal glands. A few of the small arterioles showed thickening of the intima.

One of the medium sized arteries and several of the small arteries and arterioles of the thyroid gland showed the same occluding, proliferating process of the intima that has been described for the other organs. One of the small veins in an area

showing the structure of a fetal adenoma was completely occluded by a mass of connective tissue containing a few spaces lined with endothelium. Blood pigment was not present in the mass. One small area was hyperplastic and was surrounded by a capsule of connective tissue. The follicles were otherwise normal in content of colloid.

The pancreas was normal.

The glands of the mucosa of the uterus were atrophic. The vessels showed marked thickening of the intima.

The ovary contained a moderate number of corpora albicantia. The vessels showed marked thickening of the intima.

In a skin section through an ulcer the epidermis was ulcerated, and the floor was lined by fibrous connective tissue. In one region of the floor, moderate infiltration with lymphocytes was observed. A small vessel in this region was completely obliterated by an occluding mass of connective tissue.

The intima of the aorta was slightly thickened in places.

The bone marrow and the breast did not show abnormalities.

In the inferior vena cava the lumen was filled with a mass of connective tissue continuous with the intima and penetrated by large spaces lined with endothelium. The mass of hyalinized connective tissue occluding the lumen was sparsely cellular. In one area of the mass, close to the line of the intimal endothelium, were a number of small comma-shaped nuclei. The larger vascular spaces contained few blood elements. The elastic lamella was, for the most part, intact. Recent inflammatory reaction and blood pigment were not in evidence.

The areas of destruction in the brain which were described grossly showed an advanced stage of organization, with a dense, vascular, granulation tissue. Besides this old process of organized softening, there was an acute suppurative inflammation of the leptomeninges, which extended along the meningeal covering of the blood vessels into the superficial layers of the cortex. Transverse sections through medium-sized and larger blood vessels traversing the meninges did not reveal any marked pathologic changes. In contrast with them, the sylvian artery showed a thickening of the intima with a simultaneous thickening of the elastica interna. Signs of recent inflammatory reaction were absent.

The spinal cord was normal.

The multiple foci of organized softening in the brain may be traced back to multiple thrombosis as a sequence of the endarterial changes. The endarteritis that has been described was not syphilitic. The absence of cellular infiltration and of a pronounced reaction of the connective tissue was atypical.

COMMENT

The nature of the endarterial obliterative processes has been the subject of a confusing dispute in the literature. The term "obliterative arteritis" was first introduced by Friedländer,[2] in 1876, to describe a proliferative process of the intima of arteries in inflammatory tissue as in tuberculosis. The arterial reaction is a secondary response to a surrounding inflammatory process. However, the repeated application of the phrase endarteritis obliterans or proliferans to arteriosclerotic or syphilitic processes of the extremities is so thoroughly established in

2. Friedländer: Arteritis Obliterans, Centralbl. f. d. med. Wissensch. **4**:364, 1876.

pathologic terminology that the original connotation of the phrase has been lost. Other types of endarteritis now differentiated in addition to a syphilitic endarteritis are: arteriosclerotic endarteritis, the endarteritis occurring in inflammatory tissue as in tuberculosis, and the physiologic endarterial processes such as those which occur in the ductus arteriosus and in the vessels of the uterus and of the thyroid. The morphologic variation in the reaction of the intima of a vessel is so slight that any one of a number of different varieties of pathologic or physiologic stimuli may result in the same anatomic change. The end picture does not assist in determining the etiologic factor.

In the instance reported in this paper, the sections point to a widespread obliterating process in the arterial and in part of the venous system of the body, apparently of considerable duration. Manifestations of recent inflammatory lesions were absent, as was any evidence of syphilis. Little evidence of a general arteriosclerosis of the large vessels could be found. The lesions in the vessels were apparently primarily intimal, and in the arteries consisted of a proliferative process of the connective tissue elements of the intima. With a positive Wassermann reaction and a history of repeated miscarriages, is one justified in assuming that the patient had had a syphilitic infection, and, if so, was the present pathologic picture the end-stage of a syphilitic endarteritis? In none of the sections was there any cellular reaction about the vessels such as one expects in syphilis. In none of the sections were spirochetes found. Nevertheless, this possibility cannot be excluded. It is unlikely that the condition bore more than a superficial relationship to the arteriosclerotic endarteritis of the extremities. Since arteriosclerotic endarteritis obliterans is a common condition, one would expect the disease described in this paper to occur more often.

The process, anatomically, did not show any evidence of progression, but it cannot be definitely stated that the process had been arrested. From all indications, it would seem that whatever the etiologic factors had been, they were not any longer operative. It is possible that a combination of factors was responsible, some toxin arising from the numerous abortions, as well as from syphilis, irritating the intima of the vessels.

The most marked lesions occurred in those organs which normally present a great lability of physiologic response; the uterus, thyroid, spleen and ovaries. The normal method of reducing hyperemia in these organs is by a "physiologic" obliterative endarteritis. The lesions of the vessels in the other organs in the instance described were not in any way different histologically from this physiologic reaction. It is possible that the condition was some peculiar metabolic phenomenon which manifests itself in a pathologic exaggeration of this physiologic response.

The cause of the occlusion of the inferior vena cava is not clear. The occlusion apparently was not due to thrombosis following some cause intrinsic in the vein. Pleasants,[3] in a survey of the instances of occlusion of the inferior vena cava in the literature, mentioned among the factors in thrombosis, infections such as typhoid and syphilis, and propagation from some area of infection such as the uterus. It is possible that syphilis played a rôle here, as well, though the occlusion of the uterine veins may indicate that a previous postpartum infection had been the initiating cause.

The occurrence of a low blood pressure in a person with a diffuse obliterative process of the small vessels can be explained on the basis of the subsequent coronary thrombosis. It is well known that the drop in blood pressure in instances of coronary occlusion remains permanent (if the patient recovers from the attack) in spite of a preceding hypertension.

SUMMARY

An instance of diffuse, obliterating endarteritis involving the medium sized and smaller arteries of the heart, brain, thyroid, lung, kidneys and extremities, together with an organized occluding thrombosis of the inferior vena cava in a woman, aged 47, is reported. The etiology is unknown. A single similar instance was found in the literature.

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3. Pleasants, J. H.: Obstruction of the Inferior Vena Cava with a Report of 18 Cases, Johns Hopkins Hosp. Repts. **16**:363, 1911.

THE ORIGIN OF FIBROBLASTS WITHIN AN EXPERIMENTAL HEMATOMA*

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The origin and interrelationship of the cells that comprise the connective tissue and the blood is a problem concerning which the opinions of competent investigators are at variance. Their conflicting interpretations, based on apparently sound experimental data, but data subject to personal analysis and error, confuse the literature. Excellent reviews of the current opinions of leading hematologists are available, and we shall not attempt to review such opinions except as they pertain to the subject under consideration.

That the clasmatocytes, or tissue histiocytes, and fibroblasts originate from the leukocytes of the blood is not accepted by all hematologists. Sabin, Doan and Cunningham [1] did not accept the hematogenous origin of the clasmatocyte. Maximow,[2] Lewis,[3] and others regarded the mononuclear leukocyte of the blood as the progenitor of most of the tissue macrophages. Recently, numerous studies have been published which attempt to establish the ability of the nongranular leukocyte to transform into other types of cells. Since the advent of tissue culture as a means for the growth and the direct observation of cells, studies on isolated blood have added greatly to our knowledge of the potentialities of certain of these blood cells. From cultures of leukemic blood, Awrorow and Timofejewskij [4] concluded that the lymphocytes are the stem cell from which the mononuclear leukocytes arise and that by successive division and differentiation the latter may be converted into the various other types of cells such as the macrophages, the giant cells, and even the spindle-shaped connective tissue cells. They do not use

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* From the Division of Experimental Surgery and Pathology of The Mayo Foundation.

1. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: Discrimination of Two Types of Phagocytic Cells in the Connective Tissues by the Supravital Technique, Contributions to Embryology **15-16**:125, 1923-1925.

2. Maximow, A.: Experimentelle Untersuchungen über entzündliche Neubildung von Bindegewebe, Beitr. z. path. Anat. u. z. allg. Pathol., 1902, suppl. 5, pp. 1-262.

3. Lewis, M. R.: The Formation of Macrophages, Epithelioid Cells and Giant Cells from Leucocytes in Incubated Blood, Am. J. Path. **1**:91, 1925.

4. Awrorow, P. P., and Timofejewskij, A. D.: Kultivierungsversuche von leukämischem Blute, Virchow's Arch. f. path. Anat. **216**:184, 1914.

the term fibroblast or clasmatocyte, although the cells they observed were probably of these types. Carrel and Ebeling [5] developed pure strains of the mononuclear leukocytes of the blood of adult chickens; these were kept viable for considerable periods. Cultures of pure monocytes were obtained, which differentiated into cells having the appearance of fibroblasts, although there was but slight tendency on their part to form tissues. The extent of this differentiation could be accentuated somewhat by the addition of small quantities of an embryonic extract. Lewis,[3] and Lewis and Lewis,[6] in studies of hanging drop preparations of either heart or peripheral blood of a number of vertebrates, concluded that macrophages, epithelioid cells and giant cells may arise from the blood monocytes, but that the granulocytes do not play a part in the formation of other types of cells. In these preparations they observed spindle-shaped, fibroblast-like cells, which they believed were merely elongate, multipolar macrophages; they did not find any evidence that true fibroblasts had developed from the monocytes. Fischer,[7] perhaps more than any other observer, showed a direct transformation of monocytes into connective tissue. Chick plasma containing leukocytes, together with a certain amount of embryonic extract and a fragment of muscle, was cultured and subcultured a number of times. In the fourth subculture only monocytes occurred, and some of these had become transformed into fibroblasts. In subsequent subcultures, the preparations resembled cultures of ordinary connective tissue, which later became more extensive. Accordingly, Fischer concluded that it is possible to obtain a strain of connective tissue cells from cellular elements taken directly from the blood of adult animals. Maximow [8] in studies on blood cultured with an extract of inflamed connective tissue from a rabbit, showed that the granulocytes degenerate within forty-eight hours but that the lymphocytes and the monocytes remain alive and soon differentiate into polyblasts. Even the lymphocytes hypertrophy and give rise to monocytes with typically kidney-shaped nuclei. Bloom [9] showed that the lymphocytes of the thoracic duct may transform into

5. Carrel, Alexis, and Ebeling, A. H.: Pure Cultures of Large Mononuclear Leucocytes, J. Exper. Med. **36**:365, 1922.

6. Lewis, M. R., and Lewis, W. H.: Transformation of Mononuclear Blood-Cells into Macrophages, Epithelioid Cells, and Giant Cells in Hanging-Drop Blood-Cultures from Lower Vertebrates, Contributions to Embryology **18**:95, 1926.

7. Fischer, Albert: Sur la transformation in vitro des gros leucocytes monoucleaires en fibroblasts, Compt. rend. Soc. de biol. **92**:109, 1925.

8. Maximow, A.: Ueber die Entwichlungsfähigkeiten der Blutleukocyten und des Blutgefässendothels bei entzündung und in Gewebekulturen, Klin. Wchnschr. **4**:1486, 1925.

9. Bloom, William: Transformation of Lymphocytes of Thoracic Duct into Polyblasts (Macrophages) in Tissue Culture, Proc. Soc. Exper. Biol. & Med. **24**:567, 1926-1927.

clasmatocytes, and Maximow [10] showed, in studies in vitro, that both lymphocytes and monocytes may transform into fibroblasts.

This brief review of the literature seems to indicate that some opposition to the concept of a direct transformation is still encountered; for, according to certain investigators, only fibroblast-like cells, which were temporarily spindle-shaped, developed in the cultures of the blood monocytes. These soon reverted to the macrophage type.

We wish to present here the results of certain studies which lead us to conclude that both monocytes and clasmatocytes may differentiate into fibroblasts, points concerning which there is some question. Experimentally produced hematomas of varying consecutive durations provide a satisfactory means for the study of the series of changes which transpire in blood when isolated from its normal channels. Such areas of blood are walled off from the body tissues, and the conditions which obtain are not entirely unlike those attendant on tissue culture. Temperatures are maintained constant, and circulation of new blood to the region is cut off at least during the period of study. Tissue juices that perhaps abound may correspond, in part, to the embryonic extract added to the blood when cultured in vitro. Accordingly, we were of the opinion that successive stages of such experimental hematomas would reveal the relative potency of certain of the blood cells, that is, the possibility of progressive development that may abound in them. One must grant, always, that a study based on sections has its limitations, and that sections can never be as adequate a means for the determination of cell continuity as tissue cultures, which permit relatively uninterrupted observation of a given cell.

OBSERVATIONS

The subserous spaces in the walls of the stomach and cecum of rabbits were selected as the site of the hematomas to be experimentally produced. After an aseptic injection of small quantities of autogenous blood into these areas, the animals were killed at intervals of three, five, seven, eleven, twenty-one, thirty, forty-eight and seventy-two hours. The portion of the gastro-intestinal tract, including the site of the interjection, was excised and fixed in a 10 per cent solution of formaldehyde, sectioned and stained with hematoxylin and eosin.

In the early hematomas studied, those included in the three and five hour series, the erythrocytes were intact and occasional lymphocytes, monocytes and polymorphonuclear leukocytes were present as in normal blood. Changes in any of the cells were not visible at this time. In the seven hour hematoma, however, slight changes in the organization

10. Maximow, A.: Development of Non-Granular Leucocytes (Lymphocytes and Monocytes) into Polyblasts (Macrophages) and Fibroblasts in Vitro, Proc. Soc. Exper. Biol. & Med. **24**:570, 1926-1927.

could be noted. The erythrocytes showed some signs of destruction, but most marked, probably, were the changes in the nuclei of the large monocytes. They had become more hyperchromatic, their color resembling somewhat that of the lymphocytes, and they had become slightly elongated, although still possessing their characteristic indented margin. Such changes are the first to be visible in these cells, which undergo extensive modification in later stages.

Early in the production of the hematomas the polymorphonuclear leukocytes showed signs of destruction, which is evidence that they do not play a part in the organization or transformation which ensues. The time and rate of their disappearance seem variable, for in certain stages of thirty hours' duration they were not identified, while in others they were recognized as late as seventy-two hours after the injection. This decomposition of the polymorphonuclear leukocytes has been shown to take place in tissue cultures as well. Carrel and Ebeling [9] noted the disappearance of both the granulocyte and the lymphocyte from their preparations, after the third or fourth subculture, and Maximow [8] showed that the granulocytes degenerate within forty-eight hours.

It seems evident from sections of hematomas of slightly longer duration that these cells, which rather rapidly increase in number and which possess a variety of shapes ranging from ameboid types with filiform or lobar pseudopodia to forms more distinctly spindle-shaped, are not derived from the tissue peripheral to the site of the hematoma. On the contrary, these cells are probably derivatives of the large monocytes, and possibly of the lymphocytes, which are normally within the interjected blood. Evidence is not present that these cells have migrated from the body tissue into the blood. The number of cells within the center of the hematoma is even greater than the number in the periphery, and further, the central cells do not show greater differentiation than do those nearer the periphery, a condition which might ensue were these cells of extraneous origin.

In the hematomas of fourteen and twenty-one hours' duration, certain of the cells show considerable differentiation. Most of the polymorphonuclear leukocytes have fragmented extensively; but the monocytes are more abundant and have given rise both to ameboid and to spindle-shaped cells, with somewhat elongated cytoplasmic bodies. In the thirty hour stages, the fragmented portions of the polymorphonuclear leukocytes have largely disappeared, and two rather definite types of cells are present. One of these is a large, oval cell with a single, slightly indented nucleus and clear, rather finely granular cytoplasm. This is unquestionably the blood monocyte, the clasmatocyte, or the phagocytic histiocyte of Aschoff. The other type of cell present in the thirty hour hematoma is markedly spindle-shaped, with a greatly compressed nucleus and with a cytoplasmic body now greatly attenuated,

reaching for a considerable distance among the erythrocytes. From its shape and general appearance this cell must be a fibroblast. As evidence of a peripheral origin for them is lacking, the two types of cells must be derived from the interjected blood and thus be of hematogenous origin.

In the forty-eight hour experiments these cells are further differentiated, and many of them contain numerous granules of pigment. This is particularly true of the large clasmatocytes, although the fibroblasts are frequently equally well distended with pigment (fig. 1). As fibroblasts are normally but slightly phagocytic, it is not likely that these cells have ingested these foreign particles after becoming so attentuated.

Fig. 1.—A fibroblast and a group of macrophages with ingested material in a hematoma forty-eight hours old; × 1,000.

It is more likely that the clasmatocytes which give rise to the fibroblasts had already become phagocytic before the transformation occurred. Besides these fibroblasts with inclusions of pigment, there are many others (figs. 2 and 3) which are devoid of any such phagocytosed material. Some of these are equal in size to the pigmented cells, while others are much smaller. The large fibroblasts free of pigment are probably transformed monocytes which had not phagocytosed foreign particles, while the smaller fibroblasts may be of lymphocyte origin, a conclusion not entirely verified, although Maximow [10] demonstrated in tissue culture the transformation of the small lymphocytes into macrophages.

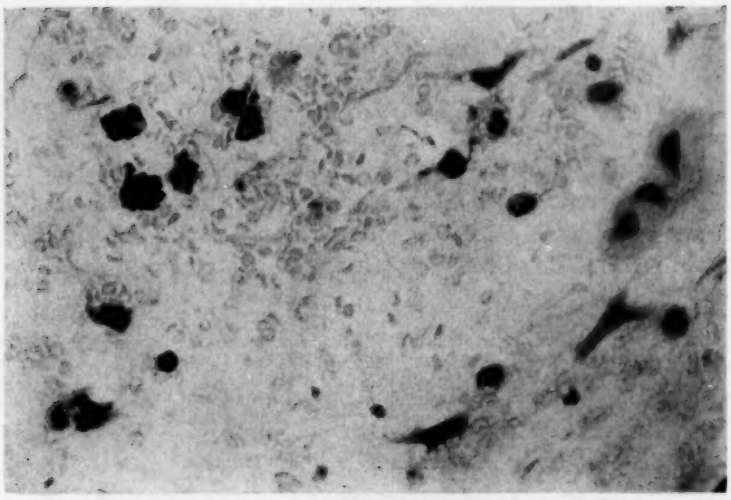

Fig. 2.—Fibroblasts with and without ingested material and a group of macrophages in a hematoma seventy-two hours old; × 475.

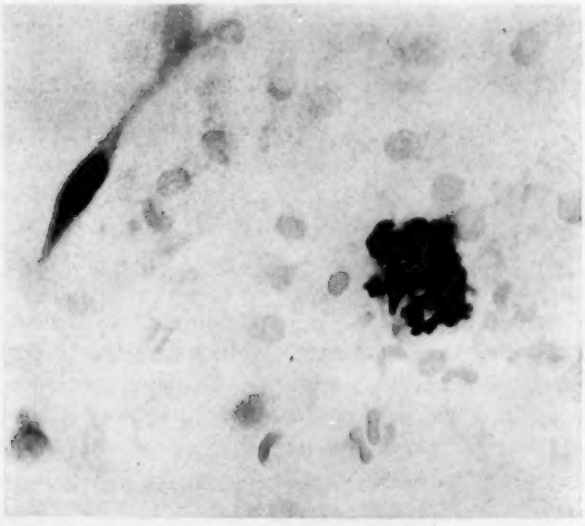

Fig. 3.—A macrophage with packed particles and a fibroblast without packed particles in a hematoma seventy-two hours old; × 1,000.

COMMENT

These observations in the early stages of a series of hematomas, artificially produced, rather clearly demonstrate the ability of certain cytologic elements of the blood to differentiate progressively into other cells. This differentiation is restricted to the monocytes and lymphocytes, for the polymorphonuclear leukocytes degenerate within forty-eight to

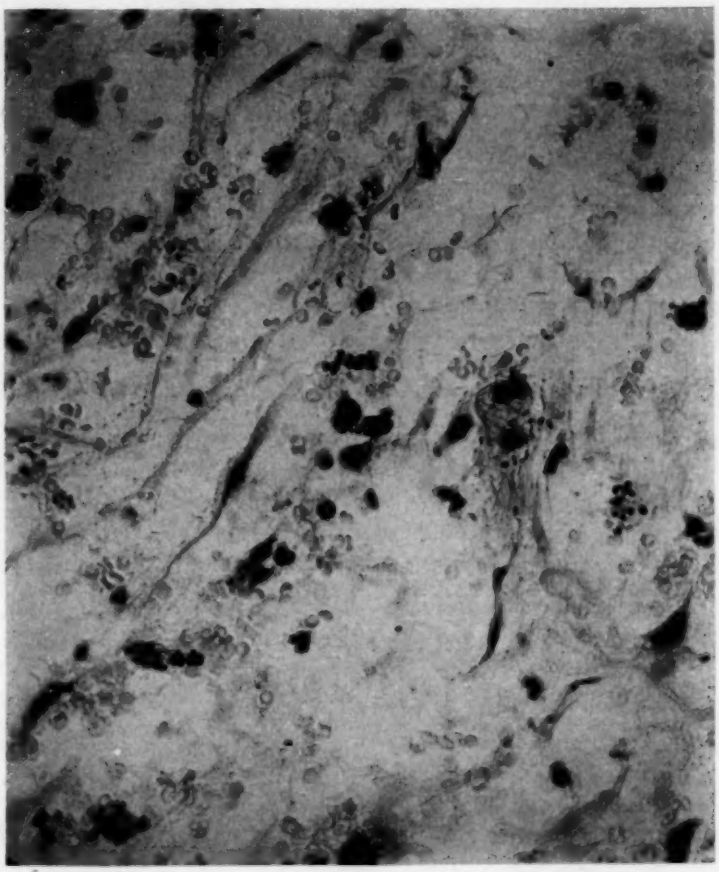

Fig. 4.—Fibroblasts with greatly attenuated processes with and without phagocytosed particles in a hematoma seventy-two hours old; × 475.

seventy-two hours, and their fragments are largely phagocytosed. The cell which becomes the most active and essentially the formative factor in the organization of the hematoma is the large blood monocyte. As early as from six to eight hours following the interjection of the blood into the subserous coat of the stomach, the nuclei of certain of these monocytes have become more ovoid, and there is some tendency to a spindle-shaped form in the cell itself. Subsequent stages in the organiza-

tion of the hematoma show further differentiation of these cells, which results in the development of two entirely distinct types, identified as the clasmatocyte or blood histiocyte and the fibroblast. In hematomas of forty-eight, sixty and seventy-two hours' duration these two types are readily identified, and in the later stages their phagocytic activity is well marked. The large, round clasmatocytes are well distended with phagocytosed pigment, and the elongated fibers of the spindle-shaped fibroblasts contain many inclusions of pigment. Besides these pigment-containing fibroblasts, there are smaller fibroblasts without pigment but with processes nearly as elongated. The large fibroblasts, containing the granules of pigment, have arisen through a transformation of the clasmatocyte, after phagocytic activity has begun. These smaller fibroblasts, on the other hand, have probably arisen in one of two ways: either as a transformation of the monocyte before phagocytosis had taken place or, as Maximow has shown, in tissue culture, directly from the larger lymphocytes. The fibroblasts which appear in these seventy-two hour hematomas are not fibroblast-like cells with only a temporary spindle form as some have described it, but rather show a definite course of progressive development from monocytes into connective tissue fibrils (fig. 4).

On the basis of these observations on a series of experimentally produced hematomas, we conclude that: (1) the blood monocytes and the lymphocytes may differentiate into other histiocytic elements; (2) the monocytes may differentiate into clasmatocytes or directly into fibroblasts; (3) the clasmatocytes may further differentiate into fibroblasts. and (4) the lymphocytes may also differentiate into fibroblasts.

SPLENIC ATROPHY WITH CALCIUM AND IRON INCRUSTATIONS (NODULAR SPLENIC ATROPHY)

REPORT OF A CASE *

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BOSTON

Nodular splenic atrophy has been only rarely recorded and is probably of infrequent occurrence. Hennings [1] and Goldberg [2] reported instances that were somewhat similar both grossly and microscopically. In the specimen to be described, as in those previously described, there was, in addition to a marked atrophy, an extensive deposition of iron pigment and lime salts.

REPORT OF CASE

Because I was unable to determine any significant relationship between the small atrophic spleen and the patient's condition, I shall give only the briefest résumé of the clinical observations.

History.—An unmarried negress, aged 21, entered the hospital complaining of pain in the legs, arms, back and neck. The illness had begun ten days before with a sore throat, headache and general malaise. On the day after admission, signs of pneumonia of the right lower lobe were made out. The clinical examination, including lumbar puncture, did not yield results of any significance, except a Wassermann reaction of one plus.[3]

Necropsy.—A complete examination six hours after death revealed an early lobar pneumonia and extensive necrosis of the liver, but no lesions of special interest except in the spleen.

The spleen weighed 10 Gm. and measured 4 by 2.7 by 1.5 cm. in its greatest diameters. It retained much the contour of a normal spleen, and on cross section it was evenly triangular. On the anterior margin, however, was a small nodule 0.5 cm. in diameter, which was less firm and retained more of the normal consistency and normal appearance of splenic tissue. The section revealed a dark slate colored surface containing strands of rather coarse fibrous connective tissue. One portion near the hilum gave a grating sensation when scraped with a knife. The splenic artery was much reduced in size, measuring on cross section approximately 2 mm. in diameter, but there was no thrombosis or occlusion of either artery or vein.

Examination of the other organs, including the brain and spinal cord, did not reveal anything of importance. A noteworthy feature, however, was that

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* From the Pathological Laboratory of the Peter Bent Brigham Hospital and Harvard University Medical School.

1. Hennings, K.: Virchows Arch. f. Path. Anat. **259**:244, 1926.

2. Goldberg, S. E.: Proc. New York Path. Soc. **26**:141, 1926.

3. Wassermann reactions in the Peter Bent Brigham Hospital are recorded as one plus and two plus.

nothing in the way of arteriosclerosis or atheroma was found in any of the arteries elsewhere in the body.

Microscopic Examination of the Spleen.—A small portion of the spleen was fixed in Zenker's solution, from which routine sections were made. The remainder of the material was fixed in formaldehyde, and this material was subsequently used for special stains, in both frozen and paraffin sections. The stains used were eosin methylene blue, hematoxylin and eosin, Roehl's hematoxylin method for staining calcium, von Kossa's and Klotz' silver method for staining calcium, ferrocyanide of potassium (Mallory) and Turnbull's blue for iron and Weigert's method for elastic tissue.

Each section represented a total cross section of the spleen. The general structural framework of the spleen was present, but the lymphoid element was

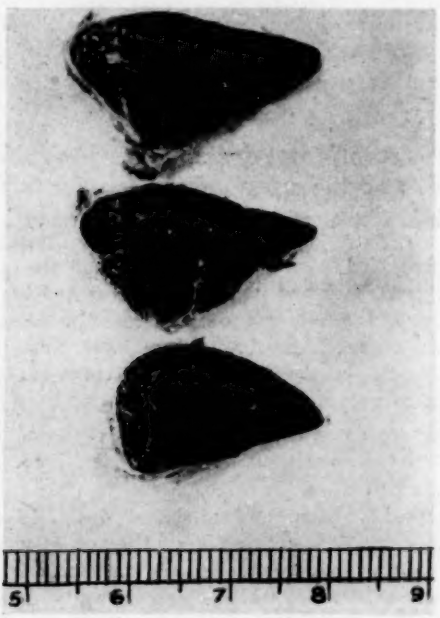

Fig. 1.—Cross sections of three portions of spleen showing marked atrophy with fibrosis and thickened walls of blood vessels; × 1.4.

almost entirely gone. Malpighian corpuscles were not found. The splenic capsule was somewhat thickened and there was an unusual prominence of the trabeculae, with here and there circumscribed areas consisting of dense fibrous connective tissue. The wide trabeculations were closely applied to one another, there being only blood, pigment, small clumps of lymphocytes and a few polymorphonuclear leukocytes and endothelial cells in the intertrabecular spaces. The vascular sinuses, where seen, were engorged with blood, and in some instances there was extravasation of blood. The blood vessels were unusually prominent and showed considerable thickening of their walls. In most instances, the small and medium-sized vessels were patent, though occasional ones were thrombosed, and these, in a few instances, showed canalization.

A considerable amount of blue and bluish-black granular deposit was seen with ordinary stains in the thickened walls of the blood vessels and in the fibrous connective tissue of the trabeculae. This substance was undoubtedly calcium, as it stained intensely with the hematoxylin, and black with the silver nitrate methods. Tissues treated with mineral acids showed complete loss of this material. When acetic acid was added to small blocks of tissue, an effervescence was noted both grossly and microscopically. Frozen sections which had been previously treated with half concentrated solution of oxalic acid to remove the iron present, and then stained as were the other sections for a brief period with silver nitrate, showed a marked thickening of the elastic lamina of the blood vessel wall, which stained black and was somewhat granular. It seems

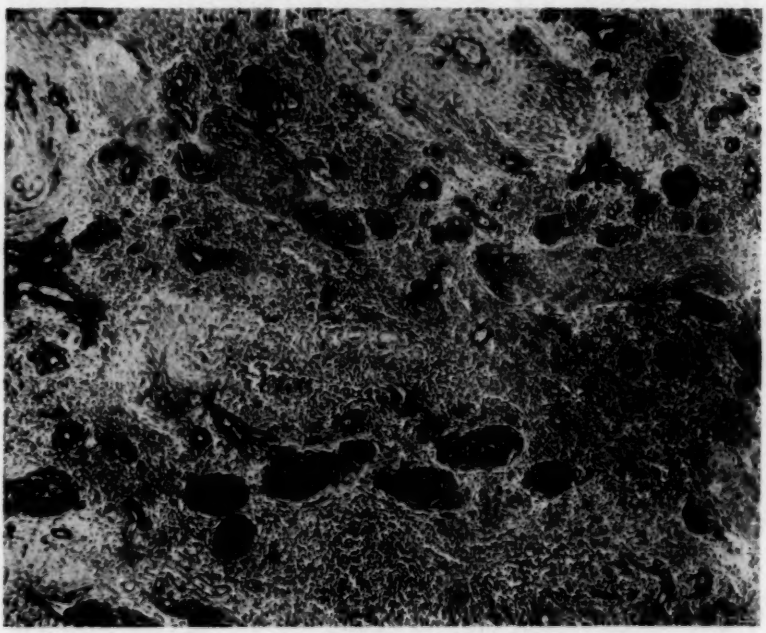

Fig. 2.—Section showing the iron and calcium deposited about blood vessels and in the fibrous and elastic tissues; stained with hematoxylin and eosin. Low power magnification.

likely from this staining reaction that the excess of lime was deposited in these laminae.

Scattered throughout the areas described was a striking amount of brownish-yellow material, which subsequently gave the characteristic iron reaction when stained by the methods mentioned. The iron could be seen both as finely divided granular particles and as an intensely blue staining material, which spread out diffusely through the connective tissue and the walls of the blood vessels. A small proportion stained as a precipitate in the pulp spaces, but, in general, it appeared to be intimately bound to the elastic and collagen structures.

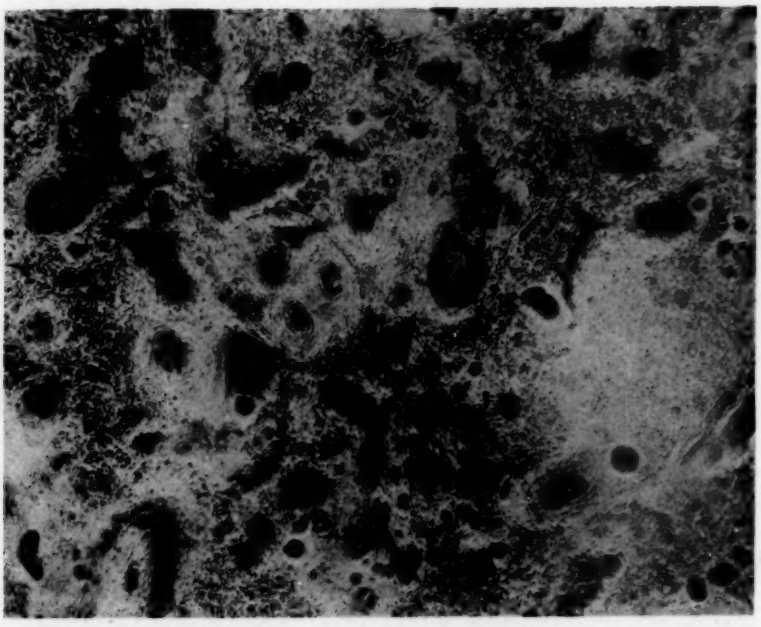

Fig. 3.—Frozen section showing the intensely staining material to be iron; stained with ferrocyanide of potassium (Mallory). Low power magnification.

Fig. 4.—Frozen section showing calcium in the walls of blood vessels and fibrous tissue; stained by von Kossa's silver method after removal of iron. Medium power magnification.

REVIEW OF LITERATURE

Sprunt,[4] in studying incrustations of calcium and iron in the elastic tissue of the spleen and the liver, concluded that degenerated, and especially calcified, elastic tissue—even more than calcification in general—seemed to possess an affinity for iron salts. The incrustations of iron occurred principally in congested or hemorrhagic areas. The distribution of the iron and the calcium in the spleen which he described follows closely that in the one here reported, which likewise showed a predominance of the iron, and the presence of iron without calcium in some areas. Watts [5] demonstrated the association of these two substances in the choroid plexus and found that certain features suggested strongly that calcification follows thrombosis of the vascular supply to the plexus. Ehrlich [6] reported that elastic fibers in the neigh-

Fig. 5.—Frozen section stained with silver nitrate, showing calcium deposited in the elastic lamina of arterial walls. Medium power magnification.

borhood of scars or infarctions have a tendency to absorb iron. He believed that the deposit of iron precedes that of calcium and found them both present in the elastic fibers and scars of splenic infarctions. Gierke [7] called attention to the physiologic association of calcium and iron in the fetus, in which the entire skeleton contains iron as far as it has been calcified, with an excess at the points of active ossification. Although the reports of incrustations of calcium and iron indicate that they are most numerous in the spleen, a similar association of the two substances has been described as occurring also in the elastic fibers of

4. Sprunt: J. Exper. Med. **45**:59, 1911.

5. Watts, J.: Deposition of Calcium Salts in Areas of Calcification, Arch. Surg. **15**:89 (July) 1927.

6. Erhlich, S.: Centralbl. f. allg. Path. u. path. Anat. **17**:177, 1906.

7. Gierke: Virchows Arch. f. path. Anat. **167**:318, 1902.

lungs which were the seat of chronic passive congestion (Bittrolff [8] and Kockel [9]). In searching for a suitable explanation of the pathologic deposition of calcium, one must first consider its possible source. Holt, LeMer and Chown [10] believed that calcium is transported as a readily soluble acid carbonate and acid phosphate, which may be converted into neutral salt by the loss of carbon-dioxide. Watts,[5] in utilizing this information, pointed out that "in places of low grade activity, such as necrotic areas, regions of fibrosis, and thrombosed vessels, there is probably little carbon-dioxide. Any fluids entering these areas will have their concentration of carbon-dioxide reduced and their calcium salts will precipitate." Hueck [11] claimed that the presence of iron in areas of calcification may be due to impurities of iron containing fixatives or to the solution of hemoglobin in fixatives which would allow its iron constituents to be deposited in calcified areas. These factors would hardly explain the entire picture as seen in the spleen in question and in those described by others, in which iron is deposited in excess of the calcium and in areas in which calcium cannot be demonstrated. Klotz [12] observed in arterial lesions that many calcified elastic fibers are seen in which iron cannot be demonstrated. On the other hand, iron-containing elastic elements were not found in the absence of calcification. Hennings' [1] report would indicate that the condition found in these spleens is produced by an increased destruction of red corpuscles after hemorrhage with deposition of hemosiderin and a peculiar disintegration of the elastic collagen material of the sclerotic wall of the blood vessel. Goldberg [2] pointed out that the excessive destruction of the red corpuscles may have been due to a toxic process or an infectious process, and that necrosis of the splenic tissue may have caused the precipitation of the iron pigment and the lime around the sclerotic areas, which had subsequently been replaced by a formation of connective tissue.

COMMENT

The interesting features of the spleen described in this paper are its extreme smallness, and the presence of iron and calcium incrustations in the walls of the blood vessels and in fibrous and elastic tissues of the trabeculae. Calcium deposit does not seem to have specificity for spleens of small size, and it has been found in various lesions of spleens as well as in those of other organs. Kaufmann [13] made note of the

8. Bittrolff: Beitr. z. path. Anat. u. allg. Path. **49**:213, 1910.
9. Kockel: Deutsches Arch. f. klin. Med. **64**:332, 1899.
10. Holt, L. E.; LeMer, V. K., and Chown, H. B.: J. Biol. Chem. **64**:509, 1925.
11. Hueck: Centralbl. f. allg. Path. u. path. Anat. **19**:774, 1908.
12. Klotz, O.: Bull. Johns Hopkins Hosp. **27**:363, 1916.
13. Kaufmann: Spezielle pathologische Anatomie, ed. 8, Berlin and Leipzig, W. de Gruyter & Company, 1928, vol. 1, p. 184.

cases of two aged women in which the spleens, weighing 19 and 10 Gm., respectively, revealed that the splenic pulp had largely disappeared and that the blood vessels and trabeculae had become correspondingly more prominent. In each instance, a brown, amorphous pigment was noted but apparently not identified. The "speckled spleen" of Feitis, recently reviewed by Hosoi,[14] does not seem to bear any definite relationship to the specimen under discussion; it is conceivable, however, that the necrotic areas of the "speckled spleen," with subsequent fibrosis, may terminate in a condition of nodular splenic atrophy. In the present case lesions of a similar nature in other organs could not be found, nor was there evidence of similar changes in the blood vessels elsewhere in the body. After consideration of the evidence at hand, it seems that because of iron in isolated fibers and in areas of considerable size without the presence of calcium the iron was of primary importance. It also seems that there was some etiologic factor, either in hemorrhage or in necrosis, or possibly in both, to cause the deposition of such a large amount of iron. While Goldberg's [2] case and the present one apparently represent the same pathologic process, there is one remarkable difference in that the former showed a nodular surface while the latter presented a surface that was smooth. It is interesting that both cases occurred in young negroes whose blood gave positive reactions in Wassermann tests.

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14. Hosoi, K.: Multiple Necrosis of the Spleen with Special Reference to the "Speckled Spleen" of Feitis, Arch. Path. **6**:26 (July) 1928.

OBSERVATIONS ON RETICULO-ENDOTHELIAL CELLS IN SEPTIC JAUNDICE*

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BROOKLYN

A male child, 11 days old, developed frequent watery stools three days before admission to the hospital. On the following day the child became jaundiced, and twenty-four hours later a small, red spot appeared in front of the left ear. The redness spread down over the shoulder, the respiration became labored and the child cried a great deal when handled.

REPORT OF CASE

History.—The infant was well nourished, deeply icteric and acutely ill; the temperature was 101 F., respirations, 40 and pulse rate, 140. The lids were closed, and somewhat edematous. The lobe of the left ear was swollen, and the area around the lobe was red and puffy. The skin back of the neck was red, and there was a definite line of demarcation between this area and the rest of the skin. The heart and the lungs were normal. The liver was palpable one and a half fingerbreadths below the free costal margin. The spleen was hard, and it was felt one fingerbreadth below the free border. The urine was normal. The blood count showed 1,860,000 red blood cells, 40 per cent hemoglobin, and 5,500 white blood cells, of which 44 per cent were polymorphonuclears and 56 per cent lymphocytes. The blood culture showed 300 colonies of *Streptococcus hemolyticus* in 1 cc. of blood. On admission, the child had been given 2,000 units of erysipelas antitoxin. Death took place after twenty-four hours.

Autopsy.—The lungs were filled with air, except in the posterior part of the right upper lobe. In this region there was a reddish-brown, soft area, about 3 inches (7.6 cm.) in diameter, which did not contain air. The same condition was found in the middle lobe of the left lung posteriorly. The heart was of medium size, and its muscle was firm. The valves were thin and smooth. The intima of the aorta was smooth. The spleen was enlarged, reddish and hard, the capsule tense; the pulp was dark red, and the trabeculae and follicles were not clearly visible. The liver was of average size, and was yellowish brown. Its structure was fairly well maintained. The gallbladder was filled with a yellowish-green bile, and the bile ducts were patent. The kidneys were of average size, the capsule stripped off readily, the surface was smooth and the structure was well maintained. The diagnosis was erysipelas, jaundice, septicemia and hypostasis of the lungs.

Microscopic examinations were made of the liver, spleen, bone marrow and suprarenal glands. On low power magnification, the structure of the liver seemed to be but slightly altered. The intertrabecular capillaries were wide and filled with some amorphous substance, which stained faintly. All the liver cells proper stained well. The veins were wide and contained much blood. High power

* Submitted for publication, Sept. 4, 1928.

* From the Department of Laboratories, United Israel Zion Hospital.

examination showed that the wide capillaries contained hardly any red blood cells. They were blocked by the enormously swollen Kupffer cells. Some of the latter were necrotic; the nuclei did not stain, and the cytoplasm was broken up. In the majority of the cells, however, the nuclei were well maintained, whereas the cytoplasm was vacuolated. Almost every cell contained a large number of bacteria which proved to be gram-positive streptococci, forming long chains (fig. 1). A few cocci were visible outside the cells, too, but their number was insignificant as compared with the large number of cocci taken up by the Kupffer cells.

There was some coagulated albuminous substance in the sinuses, particularly where the swollen Kupffer cells seemed to disintegrate.

Fig. 1.—Phagocytosed streptococci in swollen Kupffer cells. Gram stain. Leitz apochr. Oil immersion. Aperture, 2 mm. Periplane eyepiece no. 10.

But little bile pigment was visible in the liver. Most of this pigment occurred in the form of large droplets, almost the size of an erythrocyte. The droplets were found in small bile ducts.

Sections impregnated with silver after Otani's method showed the normal outline of the intracellular bile capillaries. There was no dilatation or rupture of the wall. Bile pigment was not present in these capillaries. This contrasted significantly with the bile casts which were found in some of the small bile ducts. The reticulum fibrils which lined the hepatic sinuses were poorly maintained, and apparently broken up in many places.

The most remarkable observation in the spleen under low power magnification was the scarcity and small size of the lymph follicles which consisted merely of an aggregation of small lymphocytes around little arteries. Secondary follicles were not visible. The pulp was engorged, and its cells appeared to be discrete, probably on account of the poor staining of the reticulum cell nuclei.

High power magnification showed that many of the sinus endothelial cells and also all the reticulum cells were necrotic. Some shadowy outlines of the nuclei were occasionally still visible. Other cells had completely disappeared. Many of the reticulum cells which still survived were swollen and contained a large amount of streptococci phagocytosed (fig. 2). There were also large clusters of streptococci outside the cells, but it seems that these clusters were originally intracellular and became free after the phagocytic cell had disintegrated.

There was a large amount of yellowish or greenish-yellow pigment in the spleen. The pigment was irregularly granular, or crystalline. Pigment also appeared in the form of large, round droplets similar to those described in the liver. A positive iron reaction was obtained on almost the whole pigment.

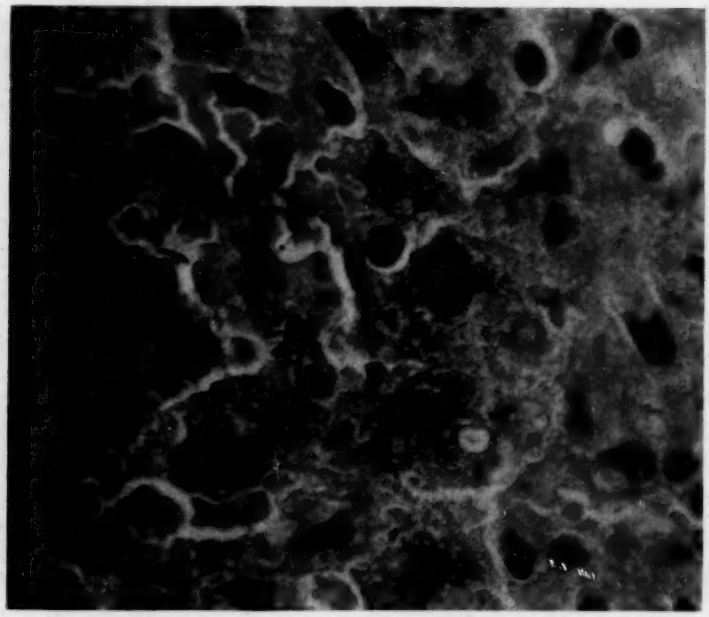

Fig. 2.—Phagocytosed streptococci in splenic reticulum cell. Hematoxylin-eosin. Leitz apochr. Oil immersion. Aperture, 2 mm. Periplane eyepiece no. 10.

The bone marrow was cellular, and there were many myelocytes particularly eosinophil in type. Other cells included mature erythrocytes, erythroblasts, lymphocytes, myeloblasts, giant cells and reticulum cells. The reticulum cells were unusually large, apparently swollen, and vacuolated. The nuclei of some of these cells were pale or did not stain. Some of the cells contained streptococci, but there were also many cocci in the meshes of the reticulum. It was noteworthy that the extracellular cocci were found in areas in which the cell nuclei did not stain any more as evidence of necrosis.

Physiologic involution of the suprarenal cortex was not completed. The medulla was scanty; its cells seemed well maintained. The cortical tissue was edematous, and the capillaries were unusually wide. The sinus endothelial cells were swollen and resembled the Kupffer cells, although they were not as bulky.

There were vacuoles in the cytoplasm, and most of the cells contained a number of streptococci. Some of the endothelial cells were seen to break up. This began with granular disintegration of the cytoplasm, whereas the nuclei faded only later (fig. 3).

COMMENT

The most remarkable observation in this case of overwhelming streptococcal infection was the enormous phagocytic activity of certain types of cells. These phagocytes included the Kupffer cells, the cells of the capillary endothelium of the suprarenals and the reticulum cells

Fig. 3.—Phagocytosed streptococci in swollen endothelial cells, reticular zone of the suprarenal cortex. Gram stain. Leitz apochr. Oil immersion. Aperture, 2 mm. Periplane eyepiece no. 10.

of the bone morrow and the spleen. This observation is noteworthy for the following reasons: 1. Phagocytosis was not incidental with these cells, as, particularly in the liver, almost all the Kupffer cells participated. 2. The phagocytic activity seemed to be specific, as other capillary endothelium, even that of the splenic sinuses, did not share in the ingestion of the cocci.

It therefore seems that, in the case reported here, the aforementioned phagocytic cells represent a functional entity which does not correspond with the general conception of the reticulo-endothelial system.

This observation is supported by other features which indicate that the Kupffer cells and reticulum cells are capable of storing electively certain substances which are not taken up by the ordinary endothelium, or by that of the splenic sinuses. Reference is made to Gaucher's disease, in which kerasin is stored electively by the same cells which are found to phagocytose streptococci in the case reported.

The failure of the splenic sinus endothelium to participate in phagocytosis is more noteworthy because the cells are known to be markedly affected in acute infectious changes of the spleen (Goldzieher [1]). They are supposed to participate in the immunologic processes of defense. Furthermore, these cells are known to phagocytose and store other material, such as fat, pigment granules and cells, especially erythrocytes.

It is important to dwell on the incongruity of the general phagocytic and storing properties of the sinus endothelia and their failure to phagocytose or store under certain pathologic conditions in which the rest of the reticulo-endothelial system is highly active. An explanation of this fact is not available; yet it seems worth while to point to the experimental results showing that storage of fat can be greatly enhanced in the reticulo-endothelial system by the injection of hormones (Goldzieher and Hirschorn [2]), whereas the sinus endothelia again appear not to be affected by such stimulation.

Another point of interest is the relationship of the reticulo-endothelial involvement to jaundice. Minute examination of the liver did not reveal any evidence of obstruction of bile ducts, and examination of the intracellular bile capillaries proved that a retention of bile had not taken place within the liver cells.

Thus, both obstructive and pleiochrome jaundice are eliminated from consideration. In typical cases of hemolytic jaundice, one expects to find erythrophagocytosis. This was not seen in the present case. It seems, therefore, that the erythrocytes dissolved in the circulation, because of the effect of streptococcal toxins, and material for the formation of bilirubin was available in excess. The elimination of the bilirubin was interfered with somewhere, and it seems from what has been said before that the obstacle was encountered prior to the entrance of bilirubin into the liver cells. The liver cells proper could hardly be held responsible, as there was not any morphologic evidence of damage to the liver cells. On the other hand, the general and severe alteration of the Kupffer cells would indicate that the function of these cells in the elimination of bilirubin was severely interfered with.

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1. Goldzieher, M. A.: The Structure of Infectious Splenic Swelling, Arch. Path. **3**:42 (Jan.) 1927.

2. Goldzieher, M. A., and Hirschorn, L.: The Reticulo-Endothelial System: III. The Influence of Hormones, Arch. Path. **4**:958 (Dec.) 1927.

It is not admissible nor intended to draw far-reaching conclusions from a single observation; nevertheless, this case seems to support the view that the liver cell is not the only factor in the formation of bilirubin, and that the Kupffer cells are actively engaged in the elimination of the bilirubin which is produced in other parts of the body. The Kupffer cells are instrumental in passing over bilirubin to the liver cells for further working up and elimination. In other words, this observation would support the view of Mann, Magath and Bollman,[3] about the formation of bilirubin outside of the liver, and would be against the contention of Melchior, Rosenthal and Licht,[4] that all other sources of possible formation of bilirubin outside of the liver are negligible.

SUMMARY

Excessive phagocytosis of streptococci was observed in a case of erysipelas with streptococcal septicemia. Kupffer cells, capillary endothelial cells of the suprarenal glands and reticulum cells of the spleen and the bone marrow participated electively in the phagocytosis. In the absence of any obstruction of the bile ducts, as well as of lesions in the liver cells, the jaundice was thought to have been caused by severe impairment of the Kupffer cells.

3. Mann, Magath and Bollman: Am. J. Physiol. **69**:393, 1924.

4. Melchior, E.; Rosenthal, F., and Licht, H.: Klin. Wchnschr. **5**:537, 1926.

HISTOCHEMICAL EVIDENCE CONCERNING THE SITE OF THE FORMATION OF BILE PIGMENT*

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The disproportionate attention given to bilirubin, which is but one of the several important constituents of bile, is no doubt due to its striking properties of color, particularly as exhibited when this pigment is retained in the body, causing the clinical condition of jaundice. Added interest in bile pigment has resulted from the knowledge, beginning with the discovery of Virchow [1] in 1847 that a pigment resembling bilirubin is found in old extravasations of blood, that the pigment in the red cells of the blood is the chief if not the only source of bilirubin.

From earliest times, the association of the liver with bile has quite naturally led to the belief that the liver manufactured the various constituents of the bile and particularly the obvious biliary pigment. Occasionally, among the older writers, there was a dissenter. Morgagni,[2] in 1760, taught that the liver merely excretes the bile brought to it, already formed, by the blood. This view, however, did not receive serious study and investigation until the latter part of the nineteenth century. At this time, the status of the hepatic cell as the normal manufacturer of biliary pigment was scarcely questioned. Since then the pendulum of thought has swung to a position where it is debated whether or not the liver cell does more than merely excrete the pigment.

Virchow [1] failed to establish that the pigment in the old extravasations of blood was identical with bilirubin, and, to differentiate it, he gave it the name of "hematoidin." This unfortunate omission in his otherwise great discovery added confusion to the subject of pigmentary metabolism and delayed the pursuit of a clue that later gave the important additional information that extrahepatic formation of bilirubin is a fact. Rich and Bumstead,[3] in 1925, finally settled the lengthy controversy by subjecting "hematoidin" obtained from old hemorrhages to a series of twenty-six physical and chemical tests and reactions that are well established as characteristic of bilirubin, and in every instance "hematoidin" reacted precisely as did a control of pure bilirubin.

In 1886, Minkowski and Naunyn [4] appeared to have offered the final answer to the question of the site of the formation of the biliary pig-

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1. Virchow, R.: Die pathologischen Pigmente, Arch. f. path. Anat. **1**:379, 1847.

2. Morgagni: De sedibus et causis morborum, epist. 36, art. 9.

3. Rich, A. R., and Bumstead, J. H.: On the Identity of Hematoidin and Bilirubin, Bull. Johns Hopkins Hosp. **36**:225, 1925.

4. Minkowski, O., and Naunyn, B.: Ueber den Icterus durch Polycholie und die Vorgänge in der Leber bei demselben, Arch. f. exper. Path. u. Pharmakol. **21**:1, 1886.

ment. They found that the intravascular hemolysis produced by arseniureted hydrogen in normal ducks and geese is regularly followed by intense jaundice, but that jaundice did not appear after such hemolysis in ducks and geese that had had their livers removed. They concluded that the liver is the site of the origin of the biliary pigment, and the principles "without the liver no formation of bile pigment" and "without the liver no jaundice" were generally established.

In 1903, Gilbert, Herscher and Posternak [5] disturbed the security of the status of the hepatic cell by reporting that normal human serum contains bile pigment. This removed the important contention that bilirubin could not be formed outside the liver because biliary pigment could not be found in the circulating blood, not even in the portal vein. Ten years later, van den Bergh and Snapper [6] applied Ehrlich's diazo-reaction to the quantitative study of bilirubin in the blood, thereby placing an extremely sensitive and delicate test in the hands of investigators. It has been repeatedly confirmed that bilirubin is present in human plasma and that it belongs to the group of "threshold substances" for the kidney and also for the liver.

In 1913, McNee [7] repeated the experiments in the dehepatization of ducks and geese that Minkowski and Naumyn had performed twenty-seven years previously and confirmed their observations. But he interpreted his results far differently. He concluded that the hepatic cells were not responsible for the production of the biliary pigment, but that the important agents were the Kupffer cells. He concluded, and later Kyes [8] confirmed his conclusions, that the biliary pigment is formed in the cells of the reticulo-endothelial system described by Aschoff,[9] the Kupffer cells being a part of that system and a particularly important part in ducks and geese because of their small spleens and small amount of bone marrow.

In that same year, Whipple and Hooper [10] attempted to exclude the liver from the circulation and found biliary pigment being formed rapidly in the blood stream following injections of hemoglobin. Rich [11]

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5. Gilbert; Herscher, and Posternak: Sur la reaction de Gmelin dans les mileux albumineux, Compt. rend. Soc. de biol. **55**:530, 1903.

6. van den Bergh, A. A. H., and Snapper: Die Farbstoffe des Blutserums, Deutsches Arch. f. klin. Med. **110**:540, 1913.

7. McNee, J. W.: Experiments on Haemolytic Icterus, J. Path. & Bact. **18**:325, 1913-1914.

8. Kyes: The Physiological Destruction of Erythrocytes in Birds, Internat. Monatschr. f. Anat. u. Physiol. **21**:542, 1915-1918.

9. Aschoff, Ludwig: Lectures on Pathology, New York, Paul B. Hoeber, 1924.

10. Whipple, G. H., and Hooper, C. W.: Icterus: A Rapid Change of Hemoglobin to Bile Pigment in the Circulation Outside the Liver, J. Exper Med. **17**:593, 1913.

11. Rich, A. R.: Experimental Studies Concerning the Site of Origin of Bilirubin, Bull. Johns Hopkins Hosp. **34**:321, 1923.

ten years later repeated these experiments, the conclusions of which had gained somewhat general acceptance, and was unable to confirm the results. He ascribed the difference in results to the variation in the completeness of the exclusion of the liver from circulation. He proved, by using Whipple and Hooper's technic, that the circulation of the liver was not completely excluded, and that therefore his work did not warrant the conclusions concerning the part the liver plays in the formation of bilirubin.

Mann, Bollman and Magath,[12] using totally dehepatized dogs, made the important contribution that bile pigment can be formed and appears in the plasma entirely independent of any action of the liver. Rich [13] confirmed this observation in a series of experiments in which he removed all the intra-abdominal viscera. Bickel [14] and Makino,[15] employing the method of removal described by Mann,[16] also noted the development of jaundice in the totally dehepatized animals. It is, therefore, well established that extrahepatic formation of bile pigment is a fact.

Evidence has not been presented that bile pigment can be formed by any cells other than those of the "reticulo-endothelial system." These cells are found particularly in the liver as Kupffer cells, and in the spleen and bone marrow as reticulo-endothelial cells, and anywhere in the body as large wandering phagocytes. Lepehne,[17] seeking to establish their importance, attempted to block this system by injecting collargol and other substances. There was some temporary success in preventing the formation of biliary pigment, but the results were inconclusive, it being apparently difficult to block this system completely. Rich and Rienhoff,[18] van den Bergh and Snapper,[19] and Kaznelson [20] found that blood

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12. Mann, F. C.; Bollman, J. L., and Magath, T. B.: Formation of Bile Pigment After Total Removal of the Liver, Am. J. Physiol. **69**:393, 1924.

13. Rich, A. R.: On the Extrahepatic Formation of Bile Pigment, Bull. Johns Hopkins Hosp. **36**:233, 1925.

14. Bickel, A.: Leberextirpation und Avitaminose in ihren Beziehungen zum Zucherstoffwechsel, Deutsche med. Wchnschr. **39**:140, 1923.

15. Makino, J.: Beiträge zur Frage der anhepatocellulären Gallenfarbstoffbildung, Beitr. z. path. Anat. u. z. allg. Path. **72**:808, 1924.

16. Mann, F. C.: Studies on the Physiology of the Liver: 1. Technic and General Effects of Removal, Am. J. M. Sc. **161**:37-42, 1921.

17. Lepehne, George; Milz, and Leber: Ein Beitrag zur Frage des hämatogenen Ikterus, zum Hämoglobin und Eisenstoffwechsel, Beitr. z. path. Anat. u. z. allg. Path. **64**:55, 1918; Untersuchungen über Gallenfarbstoff im Blutserum des Menschen, Deutsches Arch. f. klin. Med. **132**:96, 1920; Weitere Untersuchungen über Gallenfarbstoff im Blutserum des Menschen, ibid. **135**:79, 1925.

18. Rich, A. R., and Rienhoff: The Bile Pigment Content of the Splenic Vein, Bull. Johns Hopkins Hosp. **36**:431, 1925.

19. van den Bergh, A. A. H., and Snapper: Ueber anhepatische Gallenfarbstoffbildung, Berl. klin. Wchnschr. **52**:1081, 1915.

20. Kaznelson: Beitrag zur Enstehung des hämolytischen Ikterus, Wien. Arch. f. inn. Med. **1**:563, 1920.

from the splenic vein, in certain conditions, contains much more bilirubin than blood from the splenic artery or other veins.

With the reticulo-endothelial system fairly well established as a definite extrahepatic source of bilirubin, the controversy at present is over the relative rôles that the hepatic cells and the reticulo-endothelial cells of Aschoff play in the normal production of the biliary pigment. Lubarsch [21] expressed the belief that beyond all doubt the hepatic cell can produce biliary pigment. He based his belief to some extent on the finding of bilirubin within the cells of metastases of primary carcinoma of the liver. Rosenthal and Melchoir [22] urged that, the reticulo-endothelial cells being active phagocytes, the finding of biliary pigment and pigment containing iron by the side of hemoglobin indicates that all three substances could have been phagocytosed, so that catabolism from hemoglobin within the cell is not necessarily implied.

The view that the reticulo-endothelial cells are the site of origin of the biliary pigment is held at present, especially, by Aschoff,[23] Lepehne,[17] Eppinger,[24] and McNee.[25] Mann [26] thought that the liver need not be considered of importance as far as the formation of the biliary pigment is concerned. Rich [27] stated that we are unable to speak with certainty about the normal site of the formation of the biliary pigment. Nevertheless, he plainly stated that proof of the formation of the biliary pigment by the epithelial cells of the liver, under any circumstances, is lacking, and that the pigment can be formed independently of the activity of the hepatic cells. He did not consider it proved that cells of the reticulo-endothelial type can form the biliary pigment, but stated that evidence for its formation by any other type of cell or for its formation extracellularly by enzyme action, is lacking. He cited the work of Tarchanoff [28] and others to show that the hepatic cell possesses a remarkable power of taking up biliary pigment from the blood stream, and thereby invalidates the argument of Lubarsch.

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21. Lubarsch: Zur Entstehung der Gelbsucht, Berl. klin Wchnschr. **58**:757, 1921.

22. Rosenthal and Melchior: Untersuchungen über die Topik der Gallenfarbstoffbildung, Arch. f. exper. Path. u. Pharmakol. **38**:421, 1922.

23. Aschoff, L.: Ueber den Ort der Gallenfarbstoffbildung, Klin. Wchnschr. **3**:961, 1924.

24. Eppinger, Hans: Die Hepato-Lienal Erkrankungen, Berlin, Julius Springer, 1920, p. 544.

25. McNee, J. W.: Jaundice, a Review of Recent Work, Quart. J. Med. **16**:390, 1922-1923.

26. Mann, F. C.: The Site of Formation and Source of Bilirubin, Arch. Path. **2**:516 (Oct.) 1926.

27. Rich, A. R.: The Formation of Bile Pigment, Physiol. Rev. **5**:182, 1925.

28. Tarchanoff: Ueber die Bildung von Gallenpigment aus Blutfarbstoff in Thierkörper, Arch. f. d. ges. Physiol. **9**:329, 1874.

Concise résumés of the voluminous literature on the various phases of this subject can be found in excellent reviews by Rich,[27] Mann,[28] and Whipple.[29]

In reviewing the literature we felt that inadequate attention had been paid to histochemical evidence. Certain histochemical phases had been well covered, as in the work of Langhans,[30] who in 1870 studied experimental extravasations of blood and accurately observed the breaking down of hemoglobin into "hematoidin" by the large wandering phagocytes. Löwit [31] in 1889, working on the livers of normal frogs and of frogs in which experimental hemolysis had been produced, concluded that under both normal and pathologic conditions biliary pigment could be formed outside the epithelial hepatic cells within the phagocytic cells of the liver, spleen, bone marrow and circulating blood. His paper appeared shortly after the famous publication of Minkowski and Naunyn,[4] so that, since he admitted freely the formation of biliary pigment by the hepatic cells the significance of his paper was lost. Rich [32] saw red cells of the blood, in a culture from tissue, under the microscope, phagocytosed by the mesenchymal cells and transformed into bilirubin, leaving a residue containing iron. Others accurately described the breakdown of hemoglobin in the reticulo-endothelial cells in various animals and in man. But no one with whose work I am familiar, attempted in any considerable number of cases to study the histochemical evidence, using human material found at autopsy. Certain scattered observations led me to believe that such a study might throw some light on the controversy as between the reticulo-endothelial and the hepatic cells.

In various pathologic conditions associated with anemia one commonly finds bilirubin and a granular substance containing iron, called hemosiderin, side by side in the hepatic cells. Moreover, this hemosiderin is most obvious in the cells around the portal vein, while the bilirubin is most marked around the central vein. Are these pigments evidence of a transformation, within the hepatic cells, of hemosiderin into bilirubin? Are these granules containing iron merely residues after the transformation into bilirubin has taken place? Or are they stored iron, or iron in the process of excretion, or iron being built up into hemoglobin to be utilized by new red cells of the blood? And can these pigments found together in the hepatic cells be interpreted in the same way as when found together in reticulo-endothelial cells?

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29. Whipple, G. H.: The Origin and Significance of the Constituents of Bile, Physiol. Rev. **2**:440, 1922.

30. Langhans: Beobachtungen über Resorption der Extravasate und Pigmentbildung in denselben, Virchows Arch. f. path. Anat. **49**:66, 1870.

31. Löwit: Beiträge zur Lehre vom Icterus, Bietr. z. path. Anat. u. z. allg. Pathol. **4**:225, 1889.

32. Rich, A. R.: The Formation of Bile Pigment from Haemoglobin in Tissue Cultures, Bull. Johns Hopkins Hosp. **35**:415, 1924.

PROCEDURE

From seventy-one subjects who came to autopsy selected at random, blocks of tissue were taken usually from the liver and the spleen and occasionally from the kidneys, bone marrow, lungs, suprarenal gland, pancreas and lymph nodes. Some tissues were taken from material in stock, fixed in the Zenker-formaldehyde solution. The unfixed tissues were run through alcohol and cedar oil. The Zenker-fixed tissues were run through iodine-in-alcohol and mercuric-alcohol to remove the precipitate. Thin paraffin sections were stained with potassium ferrocyanide with use (after some experimentation with other stains) of Maude Abbott's high temperature modification of Mallory's technic.[33] The sections were examined for evidence of intracellular and extracellular, diffuse or granular material containing iron and brown pigment not containing iron, resembling bilirubin.

To study the effect of an increased supply of hemoglobin available for catabolism into biliary pigment, varying doses of serum from hemolyzed rabbits' blood were injected intravenously into a series of seven rabbits, various intervals of time being allowed to elapse between injections and between the final injections and death. Two rabbits were used as controls. One rabbit was killed by the intravenous injections of antihemolytic serum. One dog that died a few hours after being overtransfused, was studied. As these procedures did not yield additional information, the work will not be detailed.

OBSERVATIONS

Spleen.—Pigment was not discoverable in the organ in some cases, particularly in the spleens of young persons. Rather often there was found only a small amount of hemosiderin, but in the majority of cases a moderate amount, and in several cases of various types of anemia there was a marked amount of this pigment. Bilirubin was satisfactorily identified in small amounts in some instances.

Pigment was not found in the splenic arteries. The malpighian corpuscles were remarkably free from hemosiderin, but in a few of the pigment-laden spleens, a small amount of blue granular material was present in the reticular cells in the collar of lymphocytes. Occasionally, in the region of the connective tissue around the arteries and in the capsule and framework of the spleen, there was some diffuse blue or granular blue material. By far the greater portion of the hemosiderin was found in the large phagocytic cells of the pulp. A small amount was sometimes present within the endothelial cells lining the smaller veins. Where there had been excessive destruction of hemoglobin, the lumina of the veins usually contained considerable extracellular material. Usually, in the more moderate anemias, the pigment was largely intracellular and granular. The more rapid the catabolism of the hemoglobin, the more did the pigment containing iron tend to be diffuse instead of granular in this organ. In one case of a patient who had died after repeated transfusions of bood, red cells of the blood could be seen in various stages of disintegration in the phagocytic cells.

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33. Mallory and Wright: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924, p. 207.

Liver.—In general, the spleen contained a greater amount of hemosiderin than did the liver. The blood in the portal veins contained varying amounts of hemosiderin, the amount and type being roughly proportional and similar to that found in the splenic veins. The greatest amount of hemosiderin was found periportally in varying amounts in both the Kupffer and the hepatic cells. Apparently, the more rapid the destruction of the hemoglobin, the more pigment containing iron was found in the Kupffer cells; whereas in the more chronic anemias the greater amount of hemosiderin was found in the hepatic cells. The nearer one approached the central vein the fewer were the blue granules in both the Kupffer and the hepatic cells. In two cases with excessive destruction of hemoglobin the capillary endothelium contained occasional blue granules, and free granules containing iron between the endothelium and the rows of hepatic cells. Also, in the hyperactive cases, the hemosiderin was occasionally found in the periphery of the hepatic cell; whereas in the more severe chronic anemias, it was usually lined up on the sides of the biliary canaliculi. In the cases of extremely hyperactive catabolism of pigment, granules of hemosiderin were definitely demonstrable in the lumina of the biliary ducts. Occasionally in large biliary ducts rows of fine granules of hemosiderin were seen in the protoplasm of the cells outside the nucleus, extending from the inner to the outer border of these lining cells. The central and sublobular veins sometimes contained from a small to a moderate amount of hemosiderin, most of which was extracellular and in fine granular form. Varying amounts of hemosiderin sometimes were to be found as granules or as diffuse material scattered in the region of the framework of connective tissue and in the adventitia of the arteries. In the younger persons, more deposit of pigment was noted in the Kupffer cells than in the elderly. Diffuse pigment containing iron was not found in the hepatic cell; but in the cases of hyperactive catabolism of hemoglobin, the Kupffer cells contained much of this variety of the pigment. The Kupffer cells contained insignificant numbers of ingested red cells of the blood or particles of hemoglobin as compared with the reticuloendothelial cells of the spleen, lymph nodes and bone marrow.

Around the central vein, the bilirubin was found chiefly in the hepatic cells, while only occasionally in this region was there a moderate amount of the pigment in the Kupffer cells. When there was central necrosis and biliary stasis, the Kupffer cells around the central veins were sometimes gorged with bilirubin. The bilirubin sometimes occurred in considerable quantities in the hepatic cells with little or no hemosiderin present in the section, or there might be a considerable amount in evidence. In the latter case, there occurred an overlapping of the pigments, particularly in the midzonal area, and the pigments were sometimes found together in the same cell. The amounts of both

pigments were so considerable that they might be found together in all zones. Bilirubin in the hepatic cells was usually not associated with a demonstrable dilatation of the biliary canaliculi, although where plugs of bile were demonstrable in these canaliculi, considerable biliary pigment with plugs of pigment were found in the hepatic cells without hemosiderin. The cases of biliary stasis were associated with the largest amounts of bilirubin found in the Kupffer cells, although the Kupffer cells around the central vein might contain biliary pigment without evidence of the dilatation of a biliary duct or canaliculus. The biliary pigment was usually in the form of larger and smaller yellow brown, irregularly shaped granules and crystals, which were scattered throughout the cell except in the nucleus. Bilirubin was, of course, demonstrable in the biliary ducts, although the ducts were usually empty. Bilirubin was not demonstrable in the blood stream by this method.

Lungs.—Pigment was usually not found in the pulmonary vessels but there might be a small amount, roughly proportional to the amount found in the sublobular veins in the liver. Most of this pigment was extracellular. More pigment was found in the smaller vessels. A definite but small amount was occasionally found in the cells lining the alveoli. Some of these cells were obviously in the process of being detached from the wall of the alveoli. Most of the pigment found in the lungs was in the intra-alveolar phagocytic cells and was present in amounts comparable with the number of red cells of the blood in the alveolar spaces. These phagocytic cells appeared, in most cases, to be other than those cells derived from the lining of the alveoli. A small amount of bilirubin was identified in occasional phagocytic cells in the alveolar spaces.

Kidneys.—The persons dying of pernicious anemia were the only ones having pigment in the kidney. Of the nine cases studied, five showed more or less hemosiderin in the kidney and four were free from pigment containing iron. The iron was present chiefly in the cells lining the proximal tubules, although, in some cases, the diffuse pigment present in the lumina of the proximal tubules and in the glomerular spaces was greater than that found in the cells. Some bilirubin was demonstrable in the cells of the proximal tubules.

Bone Marrow.—Hemosiderin in the diffuse and the granular form was usually present. It was chiefly within the large reticulo-endothelial cells, which, in the pigment laden cases, appeared to be somewhat regularly spaced. A small amount of hemosiderin was found also in the endothelial cells lining the smaller sinuses. A small amount of bilirubin was identified.

Suprarenals.—Neither hemosiderin nor bilirubin was identified.

Pancreas.—Pigment that could be identified was not seen.

TABLE 1.—*Estimated Amounts of Hemosiderin (Hs) and Bilirubin (Br) Found in the Hepatic Cells and the Kupffer Cells of the Liver and in the Tissues of the Spleen, Kidney, and Other Organs of 71 Persons Coming to Autopsy Following Death from Various Causes*

Sex	Age	Hours Post-mortem	Tissues Fixed in*	Cause of Death	Hepatic Cells		Kupffer Cells		Spleen		Kidney		Other Organs
					Hs.	Br.	Hs.	Br.	Hs.	Br.	Hs.	Br.	
M	39	1	A	Acute hemolytic pancreatitis; delirium tremens	00+	00+	+	00+	....	....	....	....	(Markedly fatty liver)
F	56	3	A	Toxic adenoma	0+	++	+	00+	....	....	....	....	
F	34	1½	A	Bronchiectasis; postoperative thoracoplasty	0	+	0	000+	....	....	....	....	
M	4	26	A	Tracheotomy for papilloma of larynx; pneumonia	0	+	0	00+	....	....	....	....	
F	4	10	A	Traumatic hemorrhage into brain	00+	0	000+	0	000+	0	....	....	
M	70?	12	A	Carcinoma of bladder; perinephritic abscess	+	++	0+	00	++	0	....	....	
M	25	3	A	Postoperative appendicitis; general peritonitis	000+	++	000+	00	0	0	....	....	
F	60	?	A	Arteriosclerosis; cardiac hypertrophy and dilatation	00+	+	0	00+	00+	000+	....	....	
F	50	36	A	Malignant ulcerative endocarditis	0	++	0	0	000+	0	....	....	
M	60	6	A	Hypernephroma	0	+	000+	0	++	0	....	....	
M	70	7	A	Skull fracture	000+	++	+	000+	00+	0	....	....	
M	50	12	A	Amyotrophic lateral sclerosis; pneumonia	+++	+	++	0	+++	0	....	....	
F	15	1	A	Disseminated tuberculosis of lungs, peritoneum and meninges	00+	00+	000+	0	+++	0	....	....	
F	2	6	A	Nephritis	0	+	0	0	0	0	....	....	
M	60	5	A	Carcinoma of stomach	000+	++	0	0+	000+	0	....	....	
M	16	7	A	Postoperative brain hemorrhage; adipose genital dystrophy	0	0+	0	00+	0	0	...	....	
F	25	10	A	Chronic periostitis; abscess of brain	++	0+	0+	0	....	....	....	....	
M	67	12	A	Arteritis; cardiac hypertrophy and dilatation	+	+	+	0	++	0	....	....	Suprarenal gland, 0
M	65	½	A	Abscess of lungs	+	++	00+	0	+	0	....	....	
M	65	3	A	Arteritis; cardiac hypertrophy and dilatation	0	+	0	0	++	0	....	....	
M	55	2	A	Arteritis; cardiac hypertrophy and dilatation	0+	+	0+	0	00+	0	....	....	
M	68	1	A	Carcinoma of prostate; pneumonia	++	+	0+	0	00+	0	....	....	
F	1	12	A	Acute pericarditis; pneumonia	000+	000+	000+	0	00+	0	....	....	
M	47	5	A	Perforating gastic ulcer; pneumonia	0+	++	00+	000+	++	000+	....	....	
F	68	8	A	Carcinoma of ovary; jaundice	0+	++	++	0+	++	000+	....	....	
F	60	2	A	Sarcoma of ovary; pneumonia	+	+	+	000+	+++	0	....	....	
M	55	9	A	Toxic adenoma; pneumonia	+	+	+	00+	++	0	....	....	
F	16	15	A	Congenital syphilis; osteomyelitis; peritonitis; pneumonia	0	+	0+	0	+	0	....	....	
M	40	16	A	Perithelioma of pituitary gland; pneumonia	++	0+	+	0	+	0	....	....	
M	65	9	A	Carcinoma of panereas; jaundice	+	+	+	0	+++	000+	....	....	
F	48	17	A	Chronic nephritis; cardiac hypertrophy and dilatation	0	+	0+	0+	++	0	....	....	
M	28	1	A	Diverticulitis; peritonitis; pneumonia	+	0+	000+	0	0+	0	....	....	
M	40	2	A	Cardiac hypertrophy and dilatation; abscess of lung	0	00+	0	0+	0+	0	....	....	
F	64	2	A	Cerebral hemorrhage; pneumonia	0	+	0+	000+	++	0	....	....	
M	73	4	A	Lobar pneumonia	0	000+	0+	000+	+	0	....	....	
M	47	1	A	Acute vegetative endocarditis; pneumonia	++	++	+	0	++	0	....	....	
M	27	24	A	Chronic ulcerative colitis; peritonitis	0	+	0	0	00+	0	....	....	

F	24	1	A	Tubercular empyema; postoperative thoracoplasty	0	+	0+	0	++	0	....	....	
M	10	10	A	Vegetative endocarditis; cardiac hypertrophy and dilatation	0	+	00+	0+	0+	0	....	....	
F	53	12	A	Toxic adenoma; pneumonia	0	+	000+	00+	000+	0	....	....	
F	16	44	A	Empyema; postoperative thoracoplasty	000+	+	+	0	+	000+	....	....	
F	68	18	A	Cerebral hemorrhage; pneumonia	000+	00+	0	0	00+	0	....	....	
M	13	12	A	Leukosarcoma; intestinal obstruction	000+	0+	000+	0	000+	0	....	....	
M	56	3½	A	Portal cirrhosis; chronic myocarditis	++	000+	++	000+	+++	000+	....	....	
M	56	1	A	Cardiac hypertrophy and dilatation; pneumonia	+	++	+	0	++++	000+	....	....	
F	45	12	A	Diabetes mellitus; tendon sheath infection	+	+	0+	0	+	0	....	....	
M	5	14	A	Meningitis	+	++	+	0	++++	0	....	....	
M	70	3½	A	Carcinoma of jaw; abscess of lungs	0+	0+	+	0	+	0	....	....	
M	75	1½	A	Cardiac hypertrophy and dilatation; pneumonia	000+	++	0	00+	++	0	....	....	
F	21	1	A	Pneumonia	0	+	0	00+	+	0	....	....	
F	21	24	A	Miliary tuberculosis	0+	+	+	0	++	0	....	....	
M	45	1½	A	Postoperative carcinoma of colon; general peritonitis	0	+	0	000+	0+	0	....	....	
M	62	?	Z	Carcinoma of jaw; pneumonia	+++	+	++	0	++++	0	....	....	
M	50	8	Z	Carcinoma of stomach; pneumonia	0	++	0	000+	0	0	....	....	Kidney, lungs, 0000; suprarenal glands, pancreas, 0000; bone marrow, Hs. 000, Br. 0
M	56	11	Z	Syphilitic aneurysm; hemorrhage in lung	0+	00+	0+	000+	+	000+	....	....	Lung, 0; suprarenal gland, 0
M	45	2½	A and Z	Aleukemic leukemia; repeated transfusions	+++	0+	++	0+	++++	00+	0	0	Bone marrow, Hs. +++, Br. 000+; lung, Hs. 0, Br. 000+; lymph node, Hs. +++, Br. 00+; pancreas, 0; heart, 0
M	45	2½	A	Aleukemic leukemia; intestinal obstruction	0	++	0	000+	+	000+	0	0	Bone marrow, Hs. 00+, Br. 0; lung, 0
?	?	?	Z	Pernicious anemia	....	....	....	....	....	....	0	0	
?	?	?	Z	Pernicious anemia	....	....	....	....	....	....	+	+	
?	?	?	Z	Pernicious anemia	....	....	....	....	....	....	++	0+	
F	?	2	Z	Pernicious anemia	....	....	....	+	0	....	++	+	
M	70	½	Z	Pernicious anemia; cardiac hypertrophy and dilatation	....	....	....	....	....	....	0	0	
M	29	?	Z	Pernicious anemia; fibrous myocarditis	....	....	....	....	....	....	++	0+	
M	58	4	Z	Pernicious anemia; cardiac hypertrophy and dilatation	++++	++	+	000+	....	....	0	0	
F	67	3	Z	Pernicious anemia; rheumatic endocarditis; pneumonia	+	0+	+++	000+	....	....	+	000+	
M	65	2	A	Pernicious anemia; on Minot diet one year; pneumonia	++++	++	+	0	++	0	0	0	Suprarenal gland, 0; pancreas, 0
?	?	?	Z	Leukemia	....	....	....	....	++++	+++ Infarct	0	0	Lung, Hs. 0+, Br. 0; suprarenal gland, 0
M	3 days	?	Z	Patent interventricular septum; imperforate anus	+	0+	+++	0	++++	0	0	0	Lung, 0
M	27	1	Z	Stricture of bile ducts; jaundice	+	+++	0+	00+	++	00+	0	0	Pancreas, 0; suprarenal gland, 0
M	45	?	Z	Postoperative fibroma of brain	++	+	+	0	....	....	....	....	Lung (phag.), Hs. +++, Br. 0

* A = alcohol; Z = Zenker formaldehyde solution.

Lymph Nodes.—In the one case of a person dying after frequent transfusions, the sinuses of the leukemic lymph nodes contained many phagocytes filled with red cells of the blood and diffuse and granular hemosiderin. In these cells, an occasional granule was seen simulating bilirubin.

Little additional information was obtained from the study of rabbit tissues. Hemoglobinuria occurred in every instance in which hemolyzed serum had been injected. It was always marked enough to turn the urine a reddish black. The benzidine test was always markedly positive in these cases. In two cases urines collected from eighteen to twenty-four hours after the last injection were negative grossly and to benzidine after previous samples obtained from the same sources had been positive. In the first rabbit, a small amount of hemosiderin was found within the tubules, one cast being loaded with many granules of hemosiderin. Except for this evidence of hemosiderinuria, the animals used as controls contained quite as much pigment as did the rabbits into which hemolyzed serum had been injected. In the case of the rabbit which died following the injections of hemolytic serum, the cause of death was a complicating enteritis following a slight anemia. The day before the rabbit's death the blood count showed 5,120,000 red cells and the hemoglobin content was 80 per cent. The urine of the bladder was normal according to the benzidine test.

INTERPRETATION

The histochemical evidence pointed to the probability that the reticulo-endothelial cells, particularly those of the spleen and the bone marrow, with the Kupffer cells and the large wandering phagocytes being the chief cell reserve, break down the hemoglobin into bilirubin, and transfer it to the hepatic cells, where it is excreted into the biliary canaliculi. It could not be said with certainty that catabolism of pigment does not take place to some small degree in the hepatic cells.

COMMENT

Because the methods of histochemical study were not able, in some cases, to show the presence of pigment, I think that the breaking down of hemoglobin into bilirubin normally occurs in solution within the cells. I assume that wherever the pigments, hemosiderin or bilirubin were found, it was evidence of an excessive destruction of hemoglobin or of an interference with the excretion of it from the hepatic cells. I also assume that this visible pigment was in the same location in which the transformation normally takes place in solution or that it was in the process of transportation, the only difference being that this visible pigment was increased sufficiently above the normal to become evident in the process of fixing and staining. Most of the interpretation is based

TABLE 2.—*Estimated Amounts of Hemosiderin (Hs) and Bilirubin (Br) Found in the Various Tissues of 1 Dog That Died After Overtransfusion, and of 10 Rabbits Into Which Varying Doses of Serum from Hemolyzed Blood of Rabbits Had Been Injected at Various Intervals of Time. The Solution of Hemoglobin Used Was Approximately One Fourth of the Strength of the Serum Made Isotonic After Laking the Blood in Distilled Water*

Rabbit	Sex	Weight	Injection of Solution of Hemoglobin	Lapse of Time Between Last Injection and Autopsy	Hepatic Cells		Kupffer Cells		Spleen		Kidney		Bone Marrow		Lung		Heart and Suprarenal Gland	
					Hs.	Br.	Hs.	Br.	Hs.	Br.	Hs.	Br.	Hs.	Br.	Hs.	Br.	Hs.	Br.
1	F	2,640	5 cc. 9 a.m., 5 cc. 11 a.m., 5 cc. 1 a.m., 10 cc. 3 a.m.	30 min.	000+	00+	000+	0	+	0	000+	0	0+	0	000+	0	0	0
2	F	3,250	20 cc.	42 hrs.	0	0	0	0	+	000+	0	0	000+	0	0	0	0	0
3	M	3,520	20 cc.	24 hrs.	00+	0+	0	0	+	0	0	0	0+	0	0	0	0	0
4	F	4,120	20 cc.	1 hr.	000+	0+	0	0	0+	0	0	0	000+	0	0	0	0	0
5	F	3,120	(Bled to death; blood made up into solution of hemoglobin; used as control)	.....	0	0+	0	0	+	0	0	0	0	0	000+	0	0	0
6	F	2,260	6 cc. every 15 minutes for 10 injections	15 min.	0	+	0	000+	+	000+	0	0	0+	00+	0	0	0	0
7	F	2,600	6 cc. every 15 minutes for 12 injections	15 min.	0	+	0	000+	+	0	0	0	00+	000+	0	0	0	0
8	F	2,710	6 cc. every 15 minutes for 12 injections	15 min.	0	0+	0	0	0+	0	0	0	000+	0	0	0	0	0
9	F	2,350	(Bled three times; died).....	.....	0	0	0	0	+	0	0	0	000+	0	0	0	0	0
10	M	3,650	(Antihemolytic serum injected; died)	.....	000+	0+	0	0	0+	000+	0	0	00+	000+	0	0	0	0
Dog 1	M	.....	(Died after overtransfusion; tissues fixed in alcohol)	.....	00+	000+	000+	0	+	000+								

on the location and the type of the pigment containing iron, as the bilirubin was less satisfactorily identified.

As indicated by the presence of the pigments, the only cells involved in this catabolism of hemoglobin were the hepatic cells, the Kupffer cells, the reticulo-endothelial cells of the spleen and the bone marrow, and to a less extent, the endothelial cells of the larger sinuses of the bone marrow and the smaller veins of the spleen, the fixed phagocytes and the cells of connective tissue of the stroma of the liver and the spleen and of the adventitia of the arteries in these organs, the phagocytes of the lung alveoli, and the alveolar epithelium.

Particularly in the cases associated with the phenomena of agglutination, I saw the red blood cells ingested chiefly by the reticulo-endothelial cells of the spleen and the bone marrow and by the large wandering phagocytes, considered to be a part of the reticulo-endothelial system, that were picked up in the lymph nodes. I saw hemoglobin or parahemoglobin [34] and hemosiderin side by side in these cells; and because of the work of Rich,[32] who visualized the process in the cells of cultures of tissues, and Löwit [31] and others, I felt confident that these pigments were undergoing transformation within these cells. I felt, therefore, that normally the reticulo-endothelial cells of the spleen and the bone marrow were the most active in manufacturing biliary pigment from hemoglobin, and that in the emergencies the large wandering phagocytes became active in the catabolism of pigment. In one case with an infarct of the spleen, bilirubin was present in considerable quantities, the transformation having been completed in these cells without the usual facilities being present for the rapid removal of the bilirubin. From these reticulo-endothelial cells, the biliary pigment was probably liberated into the plasma and hurried through the Kupffer cells or the endothelial cells into the hepatic cells and excreted into the biliary canaliculi. When hemosiderin was produced in excess extrahepatically, it was liberated in the plasma or carried by the reticulo-endothelial cells to the liver, where the Kupffer cells took up the pigment and possibly transformed some of it to bilirubin, but at least, in many instances, they passed much of it on to the hepatic cells unchanged. It was not proved that the Kupffer cells picked up any bilirubin from the plasma. It might be that the endothelial cells were the avenue of approach for the bilirubin on its way to the hepatic cells.

Recent work of Jaffé and Berman [35] led me to believe that, for the foreign pigments excreted through the hepatic cells, the Kupffer cells

34. Brown, W. H.: Observations on the Origin, Distribution and Significance of Fuchsin Bodies, with Special Staining Technique, J. Exper. Med. **12**:533, 1910.

35. Jaffé, R. H., and Berman, S. L.: Functional Studies on the Relation Between Kupffer Cells and Liver Cells. Address delivered before the Chicago Institute of Medicine, 1928.

and the hepatic cells act as a unit, the stellate cells of Kupffer picking the pigment out of the blood stream and passing it on to the hepatic cells. Jaffé demonstrated this with his injections of oleokoniol, a very finely emulsified fat. In this experiment, the fine particles of fat were seen to be picked up by the stellate cells and, at a later stage, passed on to the hepatic cells, then disappearing. I take it that the hemosiderin was the foreign type of pigment, requiring the assistance of the Kupffer cells. The work of Tarchanoff [28] and others would indicate that the hepatic cells require no assistance with bilirubin. With slight obstruction of the biliary ducts, one would expect to find the bilirubin appearing first around the central vein and if it is in excess one would expect that it would involve cells nearer the portal vein and would then overflow into the Kupffer cells and later into the plasma with resultant jaundice of other tissues. My observations concurred with such a picture and not with the picture that would indicate that bile is picked up by the stellate cells from the plasma after being liberated by reticulo-endothelial cells elsewhere.

The large wandering phagocytic cells are probably the reserve of the reticulo-endothelial system. The more excessive the destruction of hemoglobin, the more prominent are these cells in the picture of the pigmentary catabolism. This might account for the difficulty that Lepehne [17] had in trying to get a complete and somewhat permanent block of the reticulo-endothelial system with injections of collargol. The phagocytes of the more fixed tissues are probably accessary in picking up some of the granular pigment containing iron. On account of their proximity to the fibers of the connective tissue, it is probable that the excess of iron diffuses slightly into the protoplasm of the connective tissue and causes the light diffuse blue staining that they commonly show in the markedly hemolytic chronic cases.

Just what the pertinent function of the endothelial cells is, as opposed to the reticulo-endothelial cells mentioned which line the sinuses of the liver, spleen and bone marrow, and the epithelial [36] cells lining the alveoli is a matter of conjecture. At least, their importance is slight.

Because of obvious technical difficulties, it was not demonstrated that the hepatic cell without the Kupffer cell can ingest hemosiderin. In all the reticulo-endothelial cells, the evidence was that the ingested hemoglobin, the ingestion of which normally takes place in solution or as a result of fragmentation (Rous [37]) or less commonly following phagocytosis of red cells of the blood, breaks down into bilirubin within

36. Fried, B. M.: The Origin of Histiocytes (Macrophages) in the Lungs: An Experimental Study by the Use of Intratracheal Injections of Vital Stain, Arch. Path. **3**:751 (May) 1927.

37. Rous, Peyton, and Robertson, O. H.: The Normal Fate of Erythrocytes, J. Exper. Med. **25**:651, 1917.

the cell and that the bilirubin is later eliminated into the plasma. In the hepatic cell, evidence was not present to indicate that hemoglobin is ingested at all, unless one assumes that the pigment containing iron represents the product of a breaking down of hemoglobin within that cell. That this is unlikely was indicated by the fact that hemosiderin in the hepatic cell was associated with hemosiderin in greater quantities in the spleen and the bone marrow. And usually there was an amount of hemosiderin in the Kupffer cells to indicate the passage of the pigment there. Furthermore, the pigment in the hepatic cells was always found to be strictly granular or irregularly crystalline, even in the cases of hyperactive hemolysis, and not diffuse. In all other locations where excessive catabolism of hemoglobin was taking place, the pigment became more diffiuse than granular or crystalline. The granular-crystalline type indicated at least temporary storage in the cell.

As some of the granular hemosiderin was found in the biliary ducts, I inferred that a threshold level exists for hemosiderin in the hepatic cell. And as there were fine granules of hemosiderin in the cells of the larger ducts, extending from one end of the cell to another, and as these areas were separated from the hepatic cells by the connective tissue of the portal canal and blood vessels, I inferred that some resorption of the hemosiderin was taking place.

The fact that the two pigments were found side by side in the hepatic cell was shown to be at least largely a matter of overlapping of stored pigment, depending on a concomitant increase in hemolysis and an interference with the excretion of biliary pigment. A case of pernicious anemia in which the patient had been treated satisfactorily over a period of a year by means of a diet high in liver, still showed a considerable deposit of hemosiderin and biliary pigment in the hepatic cells, although the bone marrow had lost its picture of pernicious anemia. This might indicate that the content of iron in the liver had been changed so that it was not easily dissociable. I did not obtain any histochemical evidence that this stored pigment is broken down into bilirubin, or that it is transformed into hemoglobin, or that as a residue of iron, or a changed pigment containing iron it remains stored in the hepatic cell.

In the case of the rabbits, I saw that the threshold of the kidney was such that large amounts of hemoglobin could be passed into the urine with little transformation into hemosiderin or bilirubin taking place in the body. That the threshold of the kidney was higher than the threshold of the hepatic cell for hemosiderin and bilirubin was indicated by the fact that hemosiderin and bilirubin were excreted into the bile before it was lost in the urine. The amount of hemosiderin found in the kidney indicated, in a given case,[38] the degree of hemosiderinuria.

38. Rous, Peyton: Urinary Siderosis, J. Exper. Med. **28**:645, 1918.

The morphologic study of the site of the formation of the biliary pigment would be made more definite if adequate methods of staining were at hand for differentiating bilirubin from melanin and hemofuscin and miscellaneous brownish granules and crystal-like substances appearing in the several tissues. Löwit,[31] in his use of the frog, was able to differentiate fairly well between hemosiderin and bilirubin and biliverdin, because, in the cases of the latter two, pigments and crystals were easily and characteristically formed in this animal. There was some question whether or not, in the use of some of the old stock Zenker material, a loss or change had occurred in the ferrous or in the biliary pigment. In most cases, at least, I felt that only inconsequential changes had resulted.

Brown [39] found that hemosiderin could be formed during autolysis of the liver. In my series of cases the number of hours elapsing between death and the autopsy (from one-half to forty-four hours) did not have any apparent effect on the formation of this pigment.

SUMMARY

A study of the literature and the material from seventy-one autopsies, with use of the ferrocyanide reaction to differentiate catabolic products of hemoglobin giving the reaction of iron from bilirubin, which does not contain iron, was undertaken in an attempt to throw additional light on the site of the formation of the biliary pigment. Hemolyzed serum was injected intravenously into seven of ten rabbits, and the tissues were studied as in the case of the human material. The liver and the spleen of an overtransfused dog were likewise examined.

It was found that the cells of the "reticulo-endothelial system" and the hepatic cells were chiefly involved in the breaking down of hemoglobin, which normally take place in solution. It was observed that the reticulo-endothelial cells of the spleen and the bone marrow were the most active in breaking down the hemoglobin, and that the Kupffer cells of the liver and the large wandering phagocytic cells were perhaps the more important reserve of the "reticulo-endothelial system." It was found that the hepatic cells have a definite threshold for hemosiderin as well as for bilirubin, and that for both of these substances the threshold is lower than that of the kidney. The occurrence of the bilirubin and the hemosiderin in the same hepatic cell was found to be an overlapping dependent on a concomitant hyperactivity of hemoglobin catabolism with the consequent storage of hemosiderin in the liver, and on an interference with the excretion of the biliary pigment from the hepatic cells. The Kupffer cells were seen to "feed" the hepatic cells with hemosiderin, but contained only bilirubin as the result of a possible

39. Brown, W. H.: Changes in the Hemosiderin Content of the Rabbit's Liver During Autolysis, J. Exper. Med. **12**:623, 1910.

transformation from hemosiderin within the cell or of an overflow of biliary pigment from the hepatic cell. The indications were that the Kupffer cell-hepatic cell combination is of little importance in the formation of biliary pigment under normal rates of pigmentary catabolism. In general, it was found that the granular crystalline type of hemosiderin indicated ferrous material that was being at least temporarily stored while the more diffuse material giving a reaction of iron was in the process of active catabolism. The granular crystalline hemosiderin was always found in the hepatic cell. Both types were found in the reticuloendothelial cells.

Evidence was not disclosed that the hepatic cell takes part in the manufacture of biliary pigment, but the possibility of a breaking down of hemoglobin within the hepatic cell has not yet been excluded.

MIGRATION OF LEUKOCYTES IN BLOOD CLOTS

I. THE INFLUENCE OF PHOSPHATES OF SODIUM AND POTASSIUM *

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The properties of leukocytes which lead to or condition their migration include the physical character of their protoplasm, their chemical constitution, their adhesiveness, probably their electrical charge and perhaps certain other factors. It is probable that essentially the same factors, with minor modifications, influence phagocytosis by leukocytes, and that what applies to the one phenomenon applies almost equally to the other. The parts played by chemotaxis and alterations of surface tension are well known, but other influences of importance must also be involved. The differences of cellular reaction in varying manifestations of inflammation are probably of significance in the understanding of these manifestations and may perhaps aid in their control and relief.

Surface tension phenomena have long been called on to explain the mobility and the phagocytic properties of leukocytes, and, for the most part, correctly. That this gives the full explanation is open to doubt. Sawtchenko [1] stated that the approach of the cell to the object to be phagocytosed is a coagglutination, even though the act of englobement is due to alterations of surface tension. Tait [2] pointed out that such approach is observable in the case of nonmobile cells and inferred that the phenomenon is one of agglutination. Whereas bacterial agglutination shows the approach and adhesion of similar particles, the mutual approach of phagocyte and phagocytic object is that of dissimilar particles. That the mechanism of approach is electrical is suggested by various experiments, but it is difficult to determine the charge of the numerous particles that may thus approach. Abrahamson [3] assumed

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1. Sawtchenko, I. G.: Sur la theorie de la phagocytose, Arch. d. sc. biol. **15:** 145, 1910.

2. Tait, J.: Capillary Phenomena Observed in Blood Cells; Thigmocytes, Phagocytosis, Amoeboid Movement, Differential Adhesiveness of Corpuscles, Emigration of Leucocytes, Quart. J. Exper. Physiol. **12:**1, 1918-1920.

3. Abrahamson, H. H.: The Mechanism of the Inflammatory Process: I. The Electrophoresis of the Blood Cells of the Horse and Its Relations to Leucocyte Emigration; II. Concerning the Adhesive Force of One Pseudopod of a Frog Leucocyte and Its Relation to Leucocyte Emigration, J. Exper. Med. **46:** 987 and 1003, 1927.

that mammalian white cells are negatively charged and stated that they move with appreciable velocity under a potential difference of 1 volt per centimeter toward the anode. It would be difficult to state that all the objects phagocytosed are positively charged, which would seem to be necessary to the hypothesis that mutual approach is a simple electrical phenomenon. The introduction in this argument of diffusion or membrane potentials seems immaterial, in view of the fact that mutual approach may be observed under extremely simple conditions in vitro. It is not altogether certain that englobement is purely physical, for Hamburger's [4] studies led him to believe that the effects are not due to surface tension but rather to alterations of the surface which influence the chemical processes within the cell body.

It has been known for years that leukocytes will migrate and adhere to solid surfaces such as glass, quartz, carbon and like substances, but will not do so in contact with greasy surfaces such as that of paraffin. The adherence and accompanying distortion, according to Tait, are a capillary phenomenon, and their conditions have been extensively studied by Fenn.[5] Fenn showed that adhesiveness to glass is best at p_H 7.8 to 8. Phagocytosis is best at p_H 7. Phagocytosis of quartz increases with the acidity down to certain limits, whereas adhesiveness of leukocytes to glass increases with the alkalinity within certain limits. Also of interest is the fact that phagocytosis of quartz particles is favored by acid solutions and phagocytosis of carbon particles by alkaline solutions. However, the adhesiveness to coal is greater than that to glass in both acid and alkaline mediums. Measurements of the carbon electrode potentials and the cataphoretic charges on the particles failed to supply an adequate explanation for these various phenomena. As Loeb [6] pointed out, the consistency of the protoplasm plays a part in adhesiveness, since surface forces must be influenced by variations in the degree of solidity of the cell.

Looking to pure science for further information, one hardly finds proof for the general assumption that cohesion, adhesion and molecular attraction in general are due to electrical phenomena. Thus, the diffi-

4. Hamburger, H. J.: Physikalisch-chemische Untersuchungen ueber Phagocyten; Ihre Bedeutung von allgemein biologischen und pathologischen Gesichtspunkt, Munich, J. F. Bergmann, 1912.

5. Fenn, W. O.: The Phagocytosis of Solid Particles, J. General Physiol. **3**:439 and 575, 1921; ibid. **5**:311, 1923; The Adhesiveness of Leucocytes to Solid Surfaces, ibid. **5**:143, 1922; Effect of Hydrogen Ion Concentration on the Phagocytosis and Adhesiveness of Leucocytes, ibid. **5**:169, 1922; The Mechanism of Phagocytosis, in Jordan, E. O., and Falk, I. S.: The Newer Knowledge of Bacteriology and Immunology, Chicago, University of Chicago Press, 1928, chap. 65.

6. Loeb, L.: Amoeboid Movement, Tissue Formation and Consisteney of Protoplasm, Science **53**:261, 1921.

culty in correlating the presumed negative charge of the leukocyte and the apparent negative charge of some of the particles phagocytosed or surfaces to which adhesion is manifest may be referred to the phenomena of molecular attraction, cohesion and adhesion, without reference to the electrical potential of the bodies concerned.

The present study is concerned with the migration of human leukocytes through a homologous blood clot to a glass surface and their adherence thereto. The method devised by Wright [7] has been modified to provide more precise control of the conditions, particularly as comparative measurements have had to be made.

METHOD OF STUDY

Wright placed agar slabs, from 1 to 2 mm. thick, from which a circular cell, 7 mm. in diameter, had been cut away, on clean microscopic slides. Blood was placed in the cell, a cover-slip was applied over the cell and the preparation was incubated. The agar was then prized off and the clot was shaken off. The slide was washed in running water and the adhering leukocytes were fixed and stained. Wright did not mention the p_H of the agar. Wolf used an agar slab as the base as well as the wall of the cell, and in certain instances added $Na_2HPO_4 .2H_2O$ to bring the agar to the neutral point to litmus.

In the present series, new glass slides were soaked in 95 per cent ethyl alcohol for not less than twenty-four hours. They were then washed in distilled water and dried with a clean towel. Strip agar was cut into pieces from 1 to 2 cm. in length, washed in running tap water for from eighteen to twenty-four hours, put through three changes of distilled water and melted in the proportion of 2.5 Gm. of dry agar to 100 cc. of distilled water. Freshly prepared agar was used in all the reported experiments. This usually titrated colorimetrically p_H 6.7 to 6.9. In order to conserve blood, we cut the cells 4 mm. in diameter. The slabs were 1.5 ± 0.05 mm. in thickness. The casting was done between two slides, separated at the ends by small pieces of slide, the thickness of which was measured by a Zeiss calibrator. The blood was taken by puncture from a finger into a paraffin chamber and mixed with a paraffined pipet, and after enough for all the cells was drawn up into the pipet, it was discharged in irregular order into the cells. This assured reasonable uniformity in the contents of all the cells. Less than one minute elapsed from the time of withdrawal to the time of filling all the cells. Technical difficulties made it unwise to use more than ten cells. The slides were warmed and kept warm on a coil of glass tube through which warm water was circulated. The temperature of the slides was from about 35 to 39 C. Incubation at 38 C. was continued in a moist chamber for forty-five minutes. In order to assure ready removal of the agar, we first placed on the slide a strip of thin tinfoil with a circular perforation 8 mm. in diameter. This allowed close approximation of the agar to the slide. After incubation, the strip of tinfoil, together with agar and cover slip and, in practically all instances, the clot also, was lifted from the slide. The slide was immersed in distilled water in a Coplin jar. After the red blood stopped diffusing, the material was fixed in saturated mercuric chloride solution, washed and stained

7. Wright, A. E.: The Study of Emigration and of the Bactericidal Effects Exerted in the Wound by Leucocytes, Lancet **1**:129, 1918. Wright, A. E., and Colebrook, L.: Technique of the Teat and Capillary Glass Tube, Constable & Company, London, 1921.

with alkaline methylene blue (methylthionine chloride, U. S. P.). Fixation with methyl alcohol and staining with Maximow's azure eosin were found to be equally satisfactory.

Incubation for forty-five minutes at 38 C. was found to be the optimal procedure. Less time did not permit as great migration, and greater time rendered difficult the distinction between polymorphonuclear and mononuclear cells.

In order to determine the influence of gravity on the migration of cells, we incubated certain series with the slides inverted. The results indicate that gravity plays but a small part in the phenomenon; almost as many cells were found adherent to the slide in the inverted position as in the usual position. This was to be expected, in view of the horizontal migration shown in the glass lath method of Wright.

A series of experiments was conducted in order to determine the amounts of $Na_2HPO_4.2H_2O$ necessary to alter in certain degrees the p_H of agar.[8] It was found that the ordinary soaking in water produces an agar, the p_H of which varies considerably. Therefore, high grade agar strips were cut in pieces from about 1 to 2 cm. in length, washed for from eighteen to twenty-four hours in running tap water, washed three times in distilled water and melted in distilled water in the Arnold sterilizer. With triply distilled water the p_H varied between p_H 6.7 and p_H 6.9. It was found that standing at room temperature for a period of two weeks increased the acidity considerably. Therefore, freshly made agar was always used. With this as a primary mixture, the amounts of $Na_2HPO_4.2H_2O$ in 5 and 10 per cent solution were added drop by drop to the agar in the proportion of so many tenths of cubic centimeters of $Na_2HPO_4.2H_2O$ solution to 9 cc. agar. The volume was brought to 10 cc. by the addition of distilled water. It was found that a fairly satisfactory curve could be established, so that by the use of specified amounts of $Na_2HPO_4.2H_2O$ the p_H could be predicted with only slight error.

The small quantities of blood employed made it difficult to determine how far the p_H of the blood is altered by the salt concentrations in the agar. The following experiment does not give a precise indication as to what happens to the blood but shows clearly that diffusion occurs. Agar cells were made in the usual way. Each cell was filled with distilled water containing from two to three times the usual amount of indicator, and a cover slip was applied. The same concentration of indicator was used in a series of tubes of different hydrogen ion concentrations. A drop of each of these was placed on a large white porcelain plate, and color comparisons were made with the contents of the cells. The indicators were bromthymol blue, phenol red and cresol red, indicated in the tables by the letters *BTB*, *PR* and *CR*. The colors in the cells began to show at the margins immediately and were completely diffused throughout the cells at room temperature in from two to three minutes. Tables 1 and 2 show readings of duplicate cells at two intervals of time.

Each of the experiments was repeated at approximately the same room temperatures, with essentially the same results. Two similar experiments with $Na_2HPO_4.2H_2O$ were performed at incubator temperature for thirty and forty-five minutes, respectively, also with essentially the same results.

Thus, diffusion from the agar into water occurs rapidly at room temperature, and within a few minutes the p_H of the water is, within the limitations of the method, the same as that of the surrounding agar. The fact is not altered by an incubator temperature of 38 C.

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8. The phosphate salts used throughout the experiments reported in this paper were of high quality and were recrystallized twice or three times in the Chemical Laboratory of the Hygienic Laboratory.

The number of leukocytes adhering to the slides with the different concentrations of a $Na_2HPO_4.2H_2O$ in the agar could be compared fairly well with the naked eye, but the extent of distribution could not be determined except with the microscope, especially because with the weaker concentrations and with the more acid hydrogen ion concentration, the leukocytes are concentrated in the middle of the circle. It was therefore necessary to measure the circles and count the cells by use of the microscope. A Zeiss ocular micrometer was calibrated against an object micrometer. The extent of two diameters of the somewhat distorted circle was measured and the cells counted in the same diameters. The large square on the ocular micrometer corresponded to a square 0.2 mm. on each side, with the tube at 15 cm., a 10× ocular and a 4 mm. objective. In the totaling of the number of

TABLE 1.—*Experiment with $Na_2HPO_4.2H_2O$ at 26 C.*

Number	p_H of Agar	p_H of Water in Cells	
		First Reading *	Second Reading
1	6.8	BTB 6.7 BTB 6.8	6.8 6.8
2	7.1	BTB 7.2 BTB 7.2	7.2 7.2
3	7.2	BTB 7.2 PR 7.2	7.2 7.3
4	7.6	PR 7.6 CR 7.5	7.6 7.5
5	8.0	CR 8.1 CR 8.0	8.1 8.0
6	8.3	CR 8.4 CR 8.4	8.4 8.4

* With BTB (brom-thymol blue), the readings were at 5 and 39 minutes; with PR (phenol red), at 2 and 30 minutes, and CR (cresol red), at 1 and 29 minutes.

TABLE 2.—*Experiment with K_2HPO_4 at 25 C.*

Number	p_H of Agar	p_H of Water in Cells	
		First Reading *	Second Reading
1	7	BTB 6.9 PR 6.9	6.9 6.9
2	7.1	BTB 7.1 PR 7.1	7.1 7.1
3	7.1	BTB 7.2 PR 7.2	7.1+ 7.1
4	7.5	PR 7.6 CR 7.5	7.6 7.5
5	8	CR 8.1 CR 8.1	8 8
6	8.3	CR 8.3 CR 8.3	8.3 8.3

* With BTB (brom-thymol blue), the readings were made at 3 and 25 minutes; with PR (phenol red), at 3 and 23 minutes, and CR (cresol red), at 3 and 19 minutes.

squares counted, one was eliminated, as it was duplicated in the crossing of the two diameters. The deduction amounts to one average square.

The protocol of a typical experiment is given in tables 3 and 4.

OBSERVATIONS

Table 4 is given in order to indicate the method in detail. Four experiments with $Na_2HPO_4.2H_2O$ were performed, including that recorded in tables 3 and 4. The results of the four experiments are summarized in table 5.

With the exception of the figure for number 3 in experiment 2, the records in table 5 indicate that within the limits of the experiments, the greater degrees of alkalinity appear to favor greater migration of leukocytes. In experiment 4, the slides were incubated upside down. It is not thought that the method is so accurate that differences of from 1,000

to 2,000 are significant, but the extremes shown are well beyond the margin of error and the intervening figures fit the conclusion.

It might be thought that the increasing number of cells with the decreasing acidity of the mixture is due to increased adhesiveness of the cells or to decreasing consistency of the clot. That the p_H of the blood clot is the same as that of the agar is not assumed, but that the blood clot

TABLE 3.—*Experiment with $Na_2HPO_4.2H_2O$*

Number	$Na_2HPO_4.2H_2O$	$Na_2HPO_4.2H_2O$ Percentage	p_H Expected	p_H Found
1	.....	....	6.7	6.8
2	5% solution 0.1 cc.	0.05	6.9	7
3	0.2 cc.	0.1	7.2	7.1
4	10% solution 0.2 cc.	0.2	7.6	7.4
5	0.5 cc.	0.5	7.8	7.8
6	1 cc.	1	8.2	8.2

TABLE 4.—*Counts of Leukocytes*

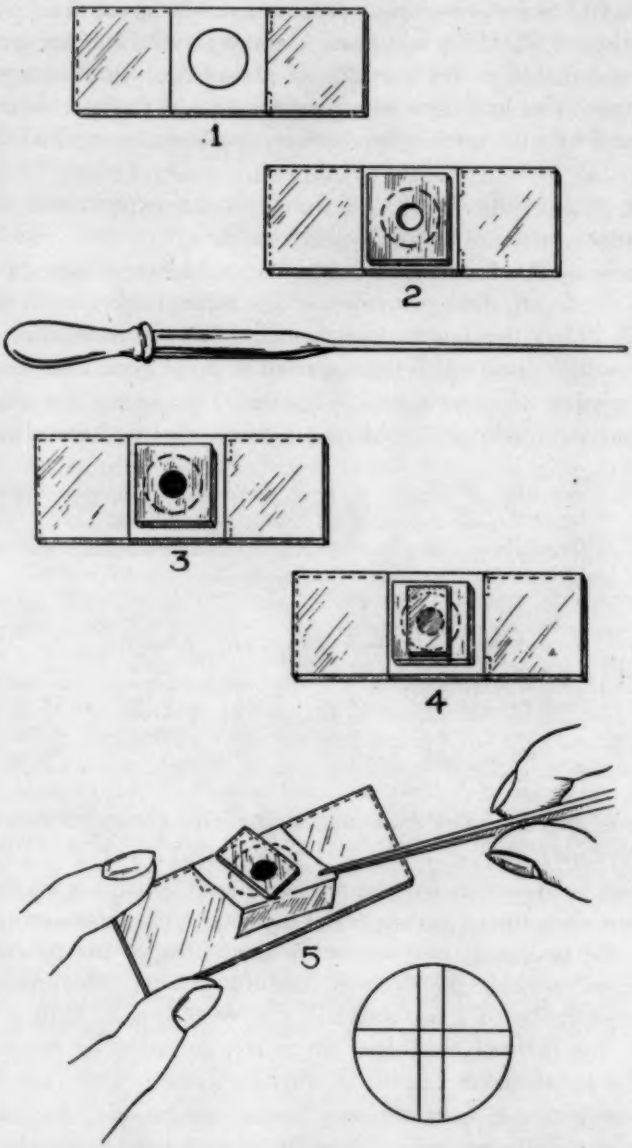
No.	Squares			Leukocytes			Per Square Millimeter	Per Circle
	Vertical	Transverse	Total	Vertical	Transverse	Total		
1	Scratched	....	....	.....	.....	.....	.....	.....
2	20.5	16.5	36	893	804	1,643	1,139	12,244
3	20.3	17.6	36.9	1,112	933	2,045	1,385	15,512
4	19.9	19.6	38.5	943	1,064	2,007	1,290	15,725
5	20	16.8	35.8	1,377	1,152	2,529	1,766	18,780
6	19	18.8	36.8	1,528	1,418	2,946	1,996	22,400

TABLE 5.—*Summary of Four Experiments Showing Influence of $Na_2HPO_4.2H_2O$ on Migration of Leukocytes in Homologous Blood Clot*

Number	Experiments							
	1		2		3		4	
	p_H	Leukocytes	p_H	Leukocytes	p_H	Leukocytes	p_H	Leukocytes
1	...	.....	6.7	11,820	...	.....	6.8	15,254
2	7	12,244	6.8	14,220	7	10,213	...	.....
3	7.1	15,512	7.4	19,400	7.1	11,063	7	20,115
4	7.4	15,725	7.5	16,760	7.4	11,573	...	.....
5	7.8	18,780	7.6	15,630	7.8	14,835	7.9	23,097
6	8.2	22,400	...	.....	8.2	19,842	8.1	22,940

by diffusion of the phosphate becomes relatively more alkaline cannot be doubted, especially in view of the rapid diffusion into water. Yet Fenn found that in the presence of serum, the adhesiveness decreases with higher degrees of alkalinity. Figures have not been obtained as to the consistency of clots at different hydrogen ion concentrations, and the determination has not been made because of the further work with potassium phosphate.

In order to ascertain whether the cation or anion was the significant factor, a series of experiments was made using K_2HPO_4 instead of



The slide with tin foil applied is shown in 1, and the agar slab superimposed in 2. In 3, the type of paraffined pipet for introducing blood into the agar cell is shown, and the cell filled with blood. In 4 the cover slip has been applied by pushing from the side. In 5 may be seen the removal of tin foil, agar, blood and cover slip. This is usually done with the slide inverted. The circle shows, within the straight lines, the diameters in which the cells are counted.

$Na_2HPO_4.2H_2O$. The 10 per cent solution of $Na_2HPO_4.2H_2O$ corresponds to 1.12 molar strength of sodium and 0.56 strength of phosphate. The solution of K_2HPO_4 was made so as to give 1.12 molar strength of potassium and 0.56 molar strength of phosphate. Preliminary experiments showed that hydrogen ion concentrations of the agar mixtures can be predicted with the same relative degree of accuracy as with the sodium salts.

Table 6 gives the results of four similar experiments in which K_2HPO_4 was used instead of $Na_2HPO_4.2H_2O$.

In three of the four experiments, the maximum migration is at p_H 7.5 and 7.6. In all, there is a more or less marked decrease in migration at p_H 8.2. Thus the favorable influence of the more alkaline reaction observed with sodium salt is not apparent with the potassium salt.

The number of experiments is too small to permit the drawing of graphs, but the information obtained is confirmed by numerous experiments in which the gross examination confirms the general impression given previously.

TABLE 6.—*Summary of Results of Four Experiments Showing Influence of K_2HPO_4 on Migration of Leukocytes in Blood Clot*

	Experiments							
	1		2		3		4	
Number	p_H	Leukocytes	p_H	Leukocytes	p_H	Leukocytes	p_H	Leukocytes
1	6.8	18,845	7	21,801	7.2	18,089	6.8	24,250
2	6.9	14,377	...	.....	7.2	18,910	7	21,850
3	7.1	19,406	7.1	28,602	7.3	20,040	7.2	16,600
4	7.5	25,119	7.5	23,206	7.6	20,476	...	.....
5	7.9	20,509	8	18,462	8	19,962	8	21,660
6	8.2	13,054	8.2	22,575	8.3	16,017	8.2	12,230

It must be apparent that the migration and adherence of the leukocytes in the presence of serum is not dependent on the concentration of anion of the two salts, nor wholly on the hydrogen ion concentration. With the sodium salt, the optimal conditions, within the limits set up, are a concentration of 1 per cent and a p_H of 8 to 8.2. With the potassium salt, the optimal conditions are a concentration of about 0.2 per cent and a p_H of about 7.5.

Thus, attention must be directed toward the cations. Jacobs [9] stated that salts of the alkaline metals increase the permeability of cells to ions; the weak bases penetrate readily. It is well known that blood corpuscles generally contain little, if any, sodium and a goodly quantity of potassium, the reverse being true of plasma; but that the leukocytes share equally with the erythrocytes in this distribution is not established.

9. Jacobs, M. H.: Permeability of the Cell to Diffusing Substances, in Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924.

Hence, there are not any grounds for assumption in regard to differential permeability of these cells to the two cations concerned. Hamburger [4] found that sodium salts are more favorable to phagocytosis than are potassium salts, but did not offer convincing evidence of a reason for the difference. Whether the difference depends on influence within the cells or in the medium is not determined at present.

If the difference is due to alterations of adhesiveness of the leukocytes, this is not per se the result of the variations in hydrogen ion concentration. Observations of the slides made with the naked eye immediately after the clot was removed would indicate that the subsequent technical operations do not significantly alter the results.

In order to test the hypothesis that the salt concentration is of greater significance than the hydrogen ion concentration, it was determined to make a solution containing essentially the same concentration of sodium and phosphate as in the 10 per cent $Na_2HPO_4.2H_2O$ buffer solution first employed, but so adjusted as to maintain the hydrogen ion concentration within a fairly narrow zone of p_H, not to exceed p_H 7.1.

This was accomplished by making a mixture of solutions of $Na_2HPO_4.2H_2O$ and NaH_2PO_4. Thus, a 10 per cent solution of $Na_2HPO_4.2H_2O$ was made as before, which was calculated to be 1.12 molar in respect to sodium and 0.56 molar in respect to phosphate. A solution of NaH_2PO_4 was made of the same molar strength as to sodium, but which, because of its composition, was necessarily 1.12 molar strength in respect to phosphate. To obtain this solution, 13.48 Gm. of anhydrous NaH_2PO_4 dried at 100 C. and kept in a desiccator, was dissolved in 100 cc. of distilled water. The final mixture was 83 cc. of a 10 per cent $Na_2HPO_4.2H_2O$ solution and 17 cc. of NaH_2PO_4 solution. This gave a buffer mixture of approximately p_H 7 and is 1.12 molar strength in respect to sodium and 0.66 molar strength in respect to phosphate. The ratio of sodium to phosphate in the $Na_2HPO_4.2H_2O$ solution is 1.12 to 0.56, and in the NaH_2PO_4 solution is 1.12 to 1.12, but since the $Na_2HPO_4.2H_2O$ predominates to a considerable extent over the NaH_2PO_4 in the mixture, the ratio of sodium to phosphate is not greatly altered from that in the original 10 per cent $Na_2HPO_4.2H_2O$ solution. It is 1.12 to 0.66 in the mixture, as compared with 1.12 to 0.56 in the original solution. The solution of the mixture of the two phosphates, therefore, is of the same molar concentration in respect to sodium as the original 10 per cent $Na_2HPO_4.2H_2O$ solution, and the ratio of sodium to phosphate is not materially altered.

Preliminary experiments with the addition of this mixture to agar showed that varying concentrations produced a p_H in the agar which remained within the limits of p_H 6.7 to 7.1. For the slight irregularities of p_H in the agar as shown in table 7, explanation is sought in the nature of agar as observed by Hoffman and Gortner,[10] who found that it contains small amounts of calcium combined with an agar acid, apparently an acid sulphuric ester, and in our experiments a small precipitate was noticed in the agar, which was presumably calcium phosphate.

10. Hoffman, N. F., and Gortner, R. A.: The Electrodialysis of Agar, J. Biol. Chem. **65**:371, 1925.

In setting up the experiments with blood, comparison was made between the effects of 0.1 per cent $Na_2HPO_4.2H_2O$ and the effects of 0.1, 0.5 and 1 per cent mixtures of the two phosphates, as shown in table 7. The method was exactly the same in all details as in the earlier experiments, and the leukocyte counts were made in the same manner.

Table 7 shows that with 0.1 per cent $Na_2HPO_4.2H_2O$ the p_H is slightly higher than with 0.1 per cent mixed salts, but in two of the three experiments the migration is about the same. A marked increase in the concentration of mixed salts, while not bringing the p_H quite up to that of the $Na_2HPO_4.2H_2O$ agar, resulted in a profound increase in the number of migrating cells. In two of the three experiments, this increased number is about the same for the 0.5 per cent and the 1 per cent buffer mixture in agar. Thus, the conclusion is plain that under the circumstances of the experiment, the concentration of the salts is of more significance than the hydrogen ion concentration.

TABLE 7.—*Comparison of Effects of $Na_2HPO_4.2H_2O$ and Mixed Phosphates; Three Experiments*

		Experiments					
		1		2		3	
Number	$Na_2HPO_4.2H_2O$	p_H	Leukocytes	p_H	Leukocytes	p_H	Leukocytes
1	0.1%	7	4,444	7.3	7,370	7.3	5,597
	Mixture						
2	0.1%	6.7	4,057	6.8	7,908	6.8	3,671
3	0.5%	6.9	15,283	7.1	8,672	7.1	14,303
4	1.0%	6.9	14,545	7.1	12,443	7.1	14,161

SUMMARY

The modification of the Wright method for studying the migration of leukocytes, here presented, gives a reasonably precise quantitative determination of migration. The important features are the use of tinfoil strips for removal of the agar, and washing in a Coplin jar instead of under running water.

When finally made into a gel, the p_H of agar is more constant after washing in running water than after soaking in still water. With a standard agar of this sort, the changes in p_H due to the addition of $Na_2HPO_4.2H_2O$ and K_2HPO_4 can be predicted with a fair degree of accuracy.

Diffusion of phosphates from agar into water, and presumably into blood clot, in the agar cells occurs promptly.

The migration of leukocytes to the walls of the cells is a true migration influenced little by gravitation. Since the largest number of leukocytes adherent to the slide is different with different concentrations of the sodium and potassium phosphates, adhesion is probably not determined entirely by hydrogen ion concentration or salt concentration.

Migration in a positive sense is also indicated by reference to the number of leukocytes adherent to all the surfaces of the cells, as compared to the content of leukocytes in the blood. It is estimated that in those instances in which migration is most marked, about 55 per cent of the leukocytes are adherent, a phenomenon that is not to be harmonized with ordinary dispersion of leukocytes in the blood or on the surfaces of the clot.

When the p_H of the agar is altered by the addition of $Na_2HPO_4.2H_2O$, the greatest migration of leukocytes occurs in the most alkaline p_H in a range between about p_H 6.8 and p_H 8.2. If K_2HPO_4 is employed instead of $Na_2HPO_4.2H_2O$, the greatest migration is in the medial p_H, namely about p_H 7.5. Thus, the hydrogen ion concentration is not the sole or even the principal factor governing migration and adhesion. When $Na_2HPO_4.2H_2O$ and $Na_2H_2PO_4$ are mixed so as to maintain a fairly constant p_H with approximately the same ratio of sodium to phosphate, as in $Na_2HPO_4.2H_2O$, the addition of increasing quantities of the mixed salts leads to a marked increase in migration of leukocytes. Thus, the concentration of salt in the agar and presumably in the blood clot is of greater significance for migration of leukocytes than is the hydrogen ion concentration within the ranges studied.

Laboratory Methods and Technical Notes

A PRACTICAL METHOD OF LABELING ANATOMIC SPECIMENS*

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In tagging gross material for identification, it is desirable that the preparation of the tag should be a simple procedure, and that the number written or stamped on the tag should be resistant to handling in the presence of such substances as are commonly used in the preservation of gross material. Of the many different methods that have been designed, one especially in use is a method in which white celluloid is employed. As this method was used in the department of pathology of the University of Maryland, the numbers easily rubbed off in handling. Therefore, a scheme was devised of treating such labels with acetone, thereby rendering the marking more permanent.

The celluloid used comes in sheets of various sizes, and may be obtained from almost any artists' supply house. It is white with one side unglazed for the purpose of marking. The tags are prepared by cutting the celluloid into pieces of the desired size and shape (a photograph trimmer may be used), and by punching a 3 mm. hole near one end. The tags are marked on the unglazed surface with a lead or an indelible pencil or by means of an automatic stamping machine. Then, the tag is dipped into acetone for a brief moment. It may then be allowed to dry or it may be placed directly in water, from which it can be taken later for attachment to the specimen.

It has been found that, if the tags are treated with acetone, the numbers do not rub off in handling. In fact, it is difficult to scratch the numbers off. Acetone, being a rapid solvent of celluloid, brings about a covering of the carbon particles with a thin coat of celluloid. Tags made according to this method are satisfactory for specimens used in teaching, which are handled much by students, and for routine autopsy material, even though the organs after the washing, following kaiserling no. 1, are placed in 95 per cent alcohol for two hours. Tags attached to specimens in kaiserling no. 3 are still in perfect condition after six months. By experiment, it has been found that these tags will dissolve in absolute alcohol and 95 per cent alcohol. In this laboratory, enough water seems to be carried over from washing, after kaiserling no. 1, to dilute the 95 per cent alcohol, so that the tags are not damaged in two hours. Tags placed in 80 per cent alcohol for several months have not shown any change in their numbers. In 10 per cent formaldehyde, they seem as permanent as though in water.

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* From the Department of Pathology, University of Maryland School of Medicine.

The method described is now regularly used in this laboratory for labeling routine necropsy material, specimens to be used in teaching, museum specimens and all surgical material. The tag is fastened to the specimen before it is placed in the museum jar. This has several obvious advantages over any label on the outside of the jar.

While this method may not be all that one could wish, yet it has the advantage of being simple and quick, and as permanent as the specimen. So far as I have been able to learn, this method has never been applied to labeling anatomic specimens, although a similar procedure may be employed in the manufacture of many celluloid articles, such as the small rule used so much in laboratories.

THE STABILITY OF GLYCERINATED ANTISHEEP HEMOLYSIN *

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While the method of Clock and Beard [1] for the preservation of antisheep hemolysin (that is, by the addition of 50 per cent by volume of chemically pure glycerol) is widely used, the literature records few observations of the duration of efficient preservation.

One of us,[2] in 1924, reported that glycerinated antisheep amboceptor that had been kept partly at room and summer temperatures, and partly in the icebox, was active and efficient after seven years.

This amboceptor has again been titered, twelve years after its original preparation (1916).

The original titer by Kolmer's method, using 0.3 cc. of complement in the dilution of 1:30, was 1:20,000. After seven years, the titer was 1:16,000. The present titer, after preservation for twelve years, is 1:10,000 with 0.3 cc. of complement in dilution of 1:30.

This observation, it is believed, will be of interest to those who desire to preserve antisheep amboceptor for long periods.

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* Submitted for publication, Oct. 17, 1928.

* From the Laboratories of the Atlantic City Hospital.

1. Clock, R. O., and Beard, S. D.: Preservation of Antisheep Hemolytic Amboceptor in Glycerol, J. Infect. Dis. **21**:404, 1917.

2. Kilduffe, R. A.: The Stability of Preserved (Glycerinated) Antisheep Hemolysin, J. Lab. & Clin. Med. **9**:651, 1924.

General Review

CELLULAR INCLUSIONS AND THE ETIOLOGY OF VIRUS DISEASES*

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A filtrable virus manifests itself usually by the changes in structure or function which it causes certain cells to exhibit. The visible effect is often restricted, so far as is known, to only one type of cell, as in the lesions of molluscum contagiosum, in which only squamous epithelium of the skin is altered. The cellular changes which different virus diseases present may vary considerably and yet possess a certain similarity. Extreme effects may be illustrated by the rapid necrosis of ectodermal cells in a herpetic infection of the rabbit's cornea, and by the slow and prolonged proliferation of cutaneous epithelial cells brought about by the virus of the common wart. In between these extremes there are many gradations from progressive degeneration of cells, consistent for long periods with at least partial integrity and vitality, as in rabies, to an extremely rapid proliferation of cells which evokes a neoplasm simulating very closely a malignant tumor.

One virus, therefore, may operate so mildly on cells that its effect manifests itself only by a slightly increased rate of proliferation, where cellular reproduction normally takes place at a certain speed, and another may stimulate cells to a violent reproductive activity. In these cases there seems to be a change in the metabolism of the cells in such a way that it is not only consistent with vitality but greatly augments the vegetative function. Another virus may alter specifically the morphology of cells, increasing their size and shape, destroying more or less their differential characteristics, suppressing or altering their specific functions. Such changes are to be interpreted as evidences of disturbed cellular activities which are indicative of injury, and cells more severely injured may die and rapidly disintegrate. That the viruses inducing these phenomena act primarily on the cells has been demonstrated in many instances by the experimental studies of several investigators on a variety of material. Thus, following an inoculation of a rabbit's cornea with the virus of variola or vaccinia a hyperplasia of corneal epithelium and the appearance of Guarnieri bodies occur before there is any evidence of inflammation in the form of a cellular

* Submitted for publication, May 12, 1928.

* From the Department of Pathology of the Vanderbilt University School of Medicine.

exudate (von Wasielewski, Rivers). In herpetic infection of the rabbit's cornea, Lipschütz has found typical intranuclear epithelial inclusions within seven hours, and before there is any other obvious reaction. The lesion of herpetic encephalitis in the rabbit is characterized by the development of typical intranuclear inclusions within ganglion cells of the affected areas before there is any manifestation whatever of inflammation, and cellular exudate occurs only after these cells have begun to disintegrate (Goodpasture and Teague). Similarly, intracellular inclusions of rabies are the first evidence of an experimental infection of the rabbit's brain (Goodpasture). The lesion of molluscum contagiosum may proceed to maturity without a reaction in the corium, and the hyperplastic nodules of fowl-pox, developing in an animal without immunity, may show no inflammatory reaction until a resistance begins to develop, terminating eventually in complete immunity, if the animal survives the infection.

It is very generally believed that viruses may alter primarily the activities and forms of cells. It is further admitted that there may be a predilection on the part of a given virus for exerting its action on a certain type of cell. This apparent specificity has led to the use of the term tropism in relation to virus infection. Some viruses are thus said to be neurotropic, namely, herpes, rabies, poliomyelitis; others, epidermotropic, as variola-vaccinia, fowl-pox, molluscum (Lipschütz).

Again it has been demonstrated that a virus may increase, and it probably always increases, in quantity locally at the site of the specific changes it effects and in intimate relation with the cells which it alters. Whether in a given instance the cells are incited to phenomenal growth or are rapidly destroyed, virus is multiplied within the lesion. The major and probably the total increase seems to take place before the cell dies, and may diminish in proportion to the disappearance of the specifically altered cells (herpetic keratitis).

Two interpretations have been offered in explanation of the multiplication of viruses, according to the interpreter's conception of the nature of these agents; namely, that they are living things and reproduce themselves by vital activity, or that they are inanimate substances and are reproduced through an interaction between themselves and the cells which they alter (Sanfelice).

Since Iwanowski discovered that the active agent of tobacco mosaic will penetrate a filter which holds back certain bacteria, and Loeffler and Frosch found the same to be true of the virus of foot and mouth disease, a great number of viruses have been shown to possess the property of filtrability through candles which are impermeable to ordinary bacteria. And those who would hold that viruses are living things may conceive of them as exceedingly minute micro-organisms,

at least in certain stages of their development, or as possessed of qualities other than size which permit of their passage through the pores of filters.

Many viruses which do not immediately kill the affected cell nor stimulate it to a rapid and continuous series of divisions, bring about peculiar structural changes within it. These changes are evidenced by the appearance of structures within the cytoplasm or within the nucleus, or both, more or less completely distinguishable from the normal cellular components, and distinctive enough to have been designated inclusions. The inclusions may be a prominent feature of those lesions induced by virus, which are constituted of a mixture of cellular proliferation and cellular degeneration, and which end spontaneously with the development of an immunity or terminate shortly in the death of the host.

It was only natural that the morphologist should have sought in the study of these intracellular bodies to find their relation to the etiologic agent at work, or to discover by means of them the peculiar activities which were taking place within cells under the influence of virus. Thus two opposed ideas have arisen, from morphologic evidence, regarding the nature of the cellular inclusions; namely, that they represent wholly or in part the virus itself, or that they consist of altered cellular constituents or products called forth by a reactive or destructive effect of the virus.

These two views are worthy of analysis with the accumulation of each additional bit of information, and especially so at the present time when there is relatively so little concentrated effort directed toward the study of virus diseases and perhaps a tendency to discount as valueless from an etiologic standpoint a considerable mass of morphologic evidence which has accumulated over a period of many years.

The inclusions of virus lesions have been studied probably most carefully, and by the largest number of investigators, in those animal diseases which are characterized by a pox or cutaneous eruption; namely, vaccinia, variola, sheep-pox, molluscum contagiosum and fowl-pox. Knowledge and theoretical considerations concerning the inclusions characteristic of the group of virus diseases have been obtained for each disease by similar processes, and a brief review of researches on the aforenamed infections will serve as a fair index of the type of investigation which has been pursued and the results obtained for the entire group of inclusions.

VARIOLA-VACCINIA

It is generally accepted that variola virus may be transformed into vaccine virus by a series of passages through the calf. The cytoplasmic inclusions incident to the two infections are similar in morphology and

are probably alike in composition. Recently it has been claimed by Gins that not only the virus of variola, but that of swine-pox, goat-pox and sheep-pox can be transformed into vaccine virus by several passages through the rabbit. This would indicate, in his opinion, that the aforementioned animal-poxes had their origin from variola. Van Heelsbergen has seemed to show that fowl-pox virus may similarly be transformed into that of vaccinia, and this view is supported by the experiments of Toyoda. The later work of Loewenthal, Kadowaki and Kondo, and of Andervont, however, seems to prove conclusively that at least the viruses of fowl-pox and of vaccinia have no such close relationship.

It is characteristic of the lesions of variola in man and in monkeys that inclusions occur within the nucleus as well as in the cytoplasm, while vaccinal lesions are characteristically associated with intracytoplasmic inclusions only. Since investigations of the two diseases have been largely parallel and the results practically identical, the two diseases are here considered together.

The inclusions of variola, although first carefully described and depicted by Guarnieri in 1892, were undoubtedly seen by Weigert as early as 1874. Guarnieri, employing the method of corneal inoculation of rabbits, first used in this connection by Straus, Chambon and Menard (1890), made a careful study of the inclusions of vaccinia. He concluded that they represent a protozoan parasite, which he named *Cytorrhyctes vacciniae*. He thought he could discover ameboid motion in the inclusions when they were observed in corneal cells suspended in tear fluid. Two modes of multiplication were described; namely, by direct division and by formation of gymnospores. The latter explanation of the mode was offered with reserve and later he discarded it, holding that these forms were degenerations.

The work of von Wasielewski (1897 and 1901) confirmed the observations of Guarnieri and supported his interpretation of the inclusions. Von Wasielewski strongly refuted the contention of those investigators who did not accept the parasitic theory. Salmon (1897), London (1898), and later Borrel (1903), regarded the inclusions as in part incorporated leukocytes. Babes maintained that they are extruded nucleoli. Ferroni and Massari considered them centrosomes. Hückel (1898) recognized the specific nature of the vaccine bodies but doubted that they represent parasites. He interpreted them rather to be a type of protoplasmic degeneration induced by a hypothetical vaccinal poison.

As opposed to these opinions, von Wasielewski concluded that the vaccine bodies are the only structures characteristic of variola and vaccinia, to be found in the lesions of the skin and the mucous membranes in these diseases. They are absent in normal skin, and in skin

injured in ways other than by the virus (numerous unsuccessful attempts had been made to reproduce identical bodies by various means).

He emphasized that whenever active lymph is placed in an epithelial abrasion of the rabbit's cornea vaccine bodies appear; under other conditions they are absent. The rapid appearance of these bodies in an avascular tissue (cornea) in the absence of inflammatory cells excludes the possibility of their origin from leukocytes. He refuted their alleged origin from epithelial cell nuclei, because of their occurrence within apparently normal cells and cells dividing by mitosis. Their origin from cell protoplasm as a result of a specific toxic action (Hückel) he held was not proved, nor disproved, but seemed improbable, because no toxic effect from vaccine lymph filtered free of virus, had been demonstrated, and because the inclusions occur in dividing cells possessing normal protoplasmic radiations.

Councilman, Magrath and Brinkerhoff (1904) were convinced of the parasitic nature of Guarnieri bodies, and Calkins (1904) in collaboration with them described in detail a complicated life cycle of the parasite, represented in the pleomorphism of the inclusions, which he classified with the *Sporozoa*. Tyzzer (1904) was likewise convinced of the parasitic nature of vaccine bodies.

After several ineffectual attempts on the part of other investigators, Negri (1905) succeeded in filtering the virus of vaccinia through Berkefeld candles IV and V, by using fresh cow lymph which had macerated several days in water at icebox temperature. Chamberland filters were impervious to the virus.

Negri emphasized that his results were not in any way to be construed as proof against the theory that Guarnieri bodies are parasites, for it might well be that in the developmental stages of such an organism filtrable phases might occur. His experiments were confirmed by Remlinger and Osman Nouri (1905).

Paschen (1906) regarded the Guarnieri bodies as a manifestation of a specific alteration of cellular material, possibly through a reaction of the cell to an invading virus, but as not the virus itself. He postulates that vaccine virus is present in vaccine lymph in enormous amounts (dilutions of 1-1000 remaining active [Prowazek]), and that it must be exceedingly small, perhaps on the border of visibility. He admits that the virus might be incorporated in an invisible form within the vaccine bodies. On staining dilute vaccine lymph from children by Giemsa's method, Paschen could demonstrate enormous numbers of very minute granules which stained uniformly. Some of them appeared split in the middle and connected by a delicate thread. No conclusions were drawn as to the nature of these bodies, although at present Paschen regards them as the etiologic agent of vaccinia (personal communication to the author.) Similar bodies were demonstrated within

the cells of vaccinal lesions by von Prowazek (1906). In 1908, Paschen reported the demonstration of the minute bodies in smears from variolous lesions. They could be demonstrated especially well in preparations stained by Loeffler's flagella method.

In 1909, Volpino described within epithelial cells of vaccinal lesions innumerable minute bodies exhibiting rapid movement. The bodies were extremely small and their motions were of different kinds. Sometimes they were simple, swift, oscillatory motions; again the movement was progressive from one point of the cell to another, so swift now and then that the moving body could no longer be accurately distinguished. At times masses of the bodies moved together from one point to another and slow changes in form and position of such groups could be seen as a result of the motion of individual constituent granules. Disintegration and rebuilding of masses of granules could be discerned as a result of separation or adhesion of individual granules with others.

These motions were considered by Volpino to be a vital phenomenon; they could be seen in cells from ten to twelve days after their removal from the host, which he believed ruled out plasma streams, for the cells were disintegrated. Von Prowazek thought the phenomenon represented merely Brownian motion. Volpino mentions that Casagrandi and Bormann had confirmed his observations.

More recently (1922) MacCallum and Oppenheimer have devised a method of separating similar extremely minute bodies in enormous numbers from vaccine lymph by the process of centrifugalizing a suspension of the virus in a fluid with a suitably adjusted specific gravity. They have found that active virus is associated with the minute bodies and is entirely absent from the fluid which is free of them. Lately (1926) Cracuin and Oppenheimer claim to have succeeded in cultivating the vaccinia granules in vitro with embryonic tissues.

Cowdry (1922) was unable to confirm the observations of MacCallum and Oppenheimer completely. By means of supravital staining he has derived evidence which convinces him that the Guarnieri bodies represent a reaction of the cell to the virus, consisting in an increase in the production of a cytoplasmic material which is normally present in minute quantities.

SHEEP-POX

Sheep-pox is a very infectious disease characterized by a nodular eruption particularly in the skin, lungs, liver and kidneys. According to Borrel, mesodermal cells are first affected and later there is an accompanying hyperplasia of epithelial cells of the tissue affected. The period of epithelial proliferation in sheep-pox is much longer than in vaccinia, and cellular destruction is less rapid. Virus is present in quantities within the nodules, and rarely demonstrable in the blood stream.

In 1901, Bosc described inclusions within the cells of the nodules, both mesodermal and epithelial, which simulate those of variola and vaccinia. He considered them to be protozoan parasites. The lesions were studied carefully by Borrel in 1903. The presence of intracytoplasmic inclusions was confirmed, but he at that time interpreted them to be due to penetration of the cells by polymorphonuclear leukocytes which degenerated there. Later he considered them, as he did vaccine bodies, to be the product of a reaction of the cell to the invasion of the virus. Borrel saw in certain cells of the lesions minute bodies measuring about one fourth of a micron in diameter, round, sharply circumscribed, arranged singly and in pairs and in chains. He cautiously suggested that these bodies might be the virus. Later, in smear preparations stained by Loeffler's flagella method, he was able to demonstrate similar extremely minute granules in enormous numbers (Lipschütz). Likewise Paschen could demonstrate such granules in smears stained by Giemsa's or Loeffler's Method (Lipschütz). They appeared similar to the granules previously found in smears from the lesions of variola and vaccinia. No one has as yet described motile granules in fresh preparations.

In 1903, Borrel succeeded in filtering the virus of sheep-pox through Chamberland candles of greater porosity than the ordinary or F filter, which was impervious to it.

MOLLUSCUM CONTAGIOSUM

The large hyaline, oval or elliptical structures which characteristically occur in or partially replace the superficial epithelial cells of the lesion of molluscum contagiosum, and which are now known as "molluscum bodies," were first described by Henderson in 1841. This was really the beginning of the study of cellular inclusions in relation to the etiology of virus diseases. Henderson regarded the bodies as parasites. In 1865, Virchow confirmed the observations of Henderson relative to the presence of molluscum bodies but considered them to be the result of a special kind of degeneration of the epidermal elements. Retzius successfully inoculated the disease on man in 1871, using material from the lesion for the inoculum, thus proving the infectious nature of the disease and demonstrating the virus content of the local lesion.

Juliusberg (1905) filtered the virus through Chamberland candles, successfully inoculating a human being with the filtrate. The filtrability of the virus was confirmed by Serra and thereafter by Wile and Kingery (1919).

The superficial nodules which constitute the lesion of molluscum have been shown by numerous investigators to consist of a local hyper-

plasia of cutaneous epithelial cells with little or no evidences of inflammation (Lipschütz, Goodpasture). The epithelial cells become greatly enlarged, especially those situated peripherally. The typical molluscum bodies occupy the most superficial zone, while the layer beneath them is composed of greatly enlarged cells, which show profound structural changes gradually leading to the transformation of their content into the typical molluscum bodies. Neisser considered these structures to be protozoa and similar to coccidia. In opposition to the parasitic view, Hansemann, MacCallum, Unna, Benda and others held that they represent a peculiar sort of cellular degeneration. The later view of von Prowazek, Lipschütz and Hartmann is that they arise as the result

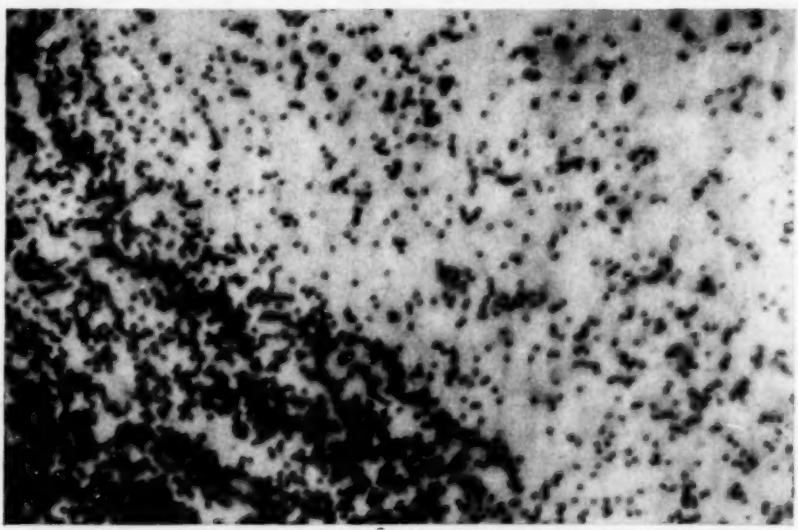

Fig. 1.—Smear from a lesion of molluscum contagiosum showing the Lipschütz bodies which compose the typical inclusions. Stain used, carbol-aniline-fuchsin; × 2000.

of a specific reaction on the part of epithelial cells to the invasion of the virus. The virus itself, according to the investigations of Lipschütz (1907), is visible and consists of minute, round, dividing bodies measuring about one fourth of a micron in diameter, which can be demonstrated in enormous numbers in fresh preparations, and in smears stained by Giemsa's stain and by Loeffler's flagella method. Lipschütz, by means of tissue sections, demonstrated these granules within the affected cells, almost filling them. They appeared in the form of compact zoogleal masses, separated into compartments, and were found only within cells. Other larger bodies of irregular size and possessing a different affinity for stains were also observed in the cytoplasm.

corresponding to similar structures, described by Benda, in the cells of fowl-pox lesions.

Da Rocha-Lima concluded that the minute bodies of Lipschütz differ from known granular elements of the protoplasm, as for example, microsomes, Altmann's granules and mitochondria. Nor are they the result of coagulation from processes of fixation, for they can be seen with great clearness in cells in the fresh condition. In fresh preparations he found the elementary bodies in compact masses within the molluscum inclusions. Examined in water the epithelial cells became somewhat swollen and then he could observe the most active molecular movement. Often, still exhibiting great activity, the small bodies would leave the cell. This molecular activity I have confirmed in observing molluscum cells suspended in physiologic sodium chloride solution at room temperature. With high magnification and ordinary illumination a most active agitation can be seen within the intracellular masses. By light compression small bits of the granular material can be squeezed from the cell. The granules remain stuck together by a transparent jelly-like material in which their active movement causes a great agitation. There was no obvious swelling of the masses when suspended in distilled water. Within cells which had been kept in saline solution in the icebox for two weeks, groups of the intracellular granules could still be seen in active motion.

Sanfelice (1918) concluded from his study of molluscum bodies that they are not parasitic but have their origin in degenerative changes, which begin in the nucleus. According to his idea, nucleoli become extruded into the cytoplasm where they are transformed into the granular masses described by Lipschütz. Lipschütz admitted a nuclear origin for the so-called Benda bodies, but recognized no genetic relation between them and the elementary bodies which constitute the main structure of the molluscum inclusions.

More recently Goodpasture (1927) maintained that the hyaline oval masses which constitute the molluscum bodies of Henderson are formed by a coalescence of the masses of intracellular granules and other intracytoplasmic material. This is brought about by desiccation as the cells are forced outward. They are not formed, in his opinion, by a sort of keratinization as suggested by Lipschütz. It further appears to him that the minute bodies, the elementary bodies of Lipschütz, are not derived from extruded nucleoli, nor from any formed cytoplasmic constituent, but are morphologically consistent with a living, filter-passing, micro-organism. They develop about and within cytoplasmic vacuoles which may be regarded as the cellular response to the presence of a living foreign body. Finally, he is of the opinion that the elementary bodies of Lipschütz are the etiologic agent of the disease.

FOWL-POX

Although one may find the statement still made that molluscum contagiosum of man and fowl-pox are the same disease manifested in different animals, there is no evidence for this beyond a certain similarity in form and manner of evolution of the lesions, and a very striking likeness in the changes which affected epithelial cells present. It has not been shown that the virus of either disease is cross-inoculable.

Fowl-pox is a specific eruptive disease of birds characterized by wart-like epithelial nodules which appear particularly on the unfeathered parts of the body and most typically about the head in spontaneous infections. The nodules consist of an epithelial hyperplasia, with little

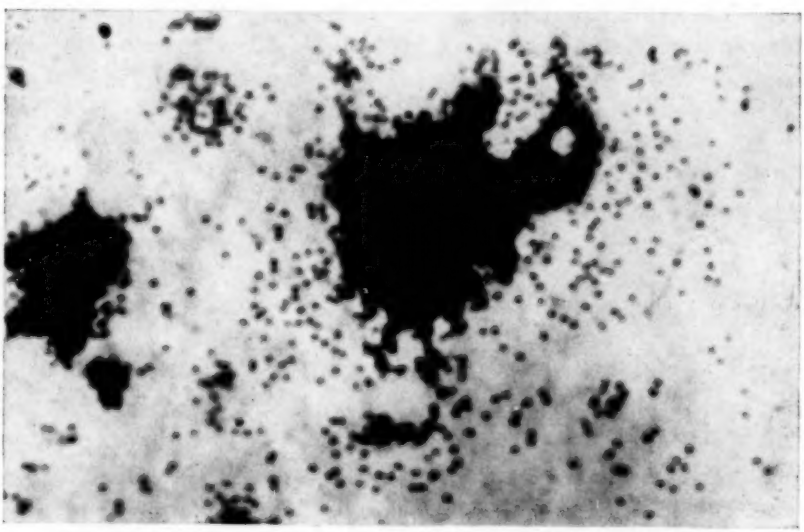

Fig. 2.—Smear from a fowl-pox lesion showing an inclusion broken up into its constituent Borrel bodies. Stain used, carbol-anilin-fuchsin; × 2000.

or no inflammation in the underlying corium in early stages. Similar lesions may occur also in the mucous membranes, which are lined by stratified epithelium. In the course of from three to five weeks immunity is acquired and the lesions become dry and encrusted. They are eventually separated by an acute inflammatory exudate beneath.

The specific cellular inclusions seem to have been first described by Rivolta in 1869. They are round, hyaline, refractive masses situated exclusively within the cytoplasm. Often they attain a size larger than that of the nucleus of the same cell. Rivolta believed them to be parasitic and classed them with the *Gregarina*. The parasitic theory was maintained by Mingazzini, Sanfelice and others. Polowinkin,

Apolant, and lately Ludford and Findlay have considered the inclusions to be the result of cellular degeneration. Michaelis (1903) observed that they give reactions for fat with scarlet red and with osmic acid. Apolant found bodies in the affected cells which he considered to be extruded nuclear particles, but the fowl-pox inclusions proper, he thought, have an entirely cytoplasmic origin.

In 1902, Marx and Sticker succeeded in filtering the virus through Berkefeldt candles, but found it to be held back by the Chamberland F filter. They therefore disregarded the structures previously described as etiologic agents and definitely placed the virus among the filter-passers.

Borrel introduced a new morphologic conception of the virus of fowl-pox and other pox-like diseases when he discovered (1904) in smears from the lesion, stained by Loeffler's flagella method, myriads

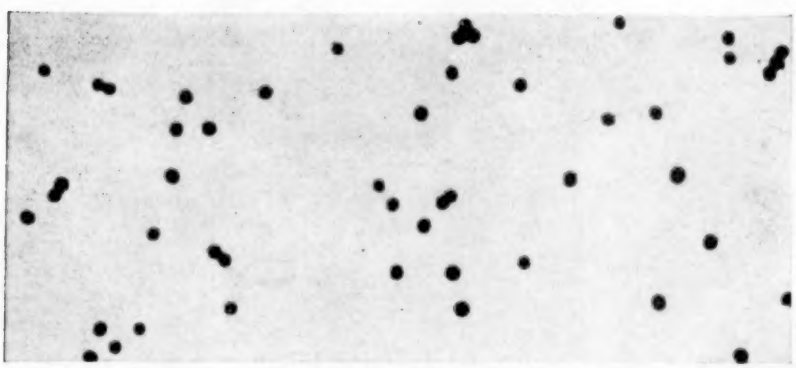

Fig. 3.—Smear from a culture of *Staphylococcus aureus,* stained and magnified the same as the smears in figures 1 and 2, for comparison. The photomicrographs were taken from smears prepared all in the same way. They were made with panchromatic plates and a B filter.

of minute (¼ micron), uniform, coccoid bodies arranged singly, in pairs, and in short chains. That these small structures, more diminutive than any known bacteria, represent the virus of fowl-pox, was accepted by Burnet, Lipschütz, von Prowazek and others. Burnet considered three hypotheses possible in explanation of the the large cytoplasmic inclusions, namely: (1) they are parasitic; (2) they are the products of secretion or degeneration of the cell brought about through the action of the unknown virus; (3) they enclose, enveloped in a substance of cellular secretion or degeneration, a micro-organism, whose form accords with the acquired facts of abundance and filtrability of the virus. Burnet's own investigations led him to adopt the last hypothesis. The enormous numbers of the micrococci, which he considered the

Borrel bodies to be, accords, in his opinion, with the extraordinary abundance of the virus. The fact that they occur in masses might account for the difficulty with which they are filtered, and perhaps for the unusual resistance to various external conditions which is characteristic of the virus. Loewenthal, however, still considered the inclusions typical of this and related diseases to be the product of cellular reaction; and Sanfelice, abandoning his idea of a blastomycotic etiology, discarded entirely the parasitic theory and supposed the disease to be due to a nonliving toxic substance which is reproduced as a result of the cellular injury which it induces. The inclusions are, accordingly, only manifestations of a degenerative change within the injured cells.

Recently it was shown by Goodpasture (1928) that the inclusions of fowl-pox can be easily removed from the cells, and when suspended in saline solution they appear as compact, hyaline, refractive bodies. When washed and suspended in distilled water they can be observed to swell rapidly becoming transformed into a number of globular compartments which tend to coalesce. After the swelling has continued for from one-half to one hour and the globules have reached a considerable size one can see, with the oil immersion lens and ordinary illumination, an extremely rapid agitation taking place within the globule. On careful observation it can be seen that this motion is due to the vibration of great numbers of minute uniform bodies, which are considered to be identical with the granules of Borrel, and the etiologic agent of the disease. When the swollen bodies are returned to saline solution they rapidly shrink through the loss of water and again become dense and hyaline. If a single large inclusion be crushed or smeared on a slide and suitably stained,[1] the minute bodies are clearly demonstrated incorporated by a hyaline matrix, which does not have the appearance of microbic capsules which have coalesced, but that of a more independent and voluminous material in which the granules are actually suspended.

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1. The minute bodies of fowl-pox and of molluscum contagiosum can be clearly demonstrated by staining a thin smear from a lesion in the following manner.

Dry the smear and fix by gently heating in a flame.

(1) Mordant for one minute in ¼ per cent potassium permanganate.

(2) Wash thoroughly in water.

(3) Stain for one minute in the following solution:

Alcohol 30 per cent	100 cc.
Basic fuchsin	1 Gm.
Phenol (crystalline)	1 cc.
Aniline oil	1 cc.

(4) Wash thoroughly in water.

(5) Blot and dry. Observe with oil immersion objective.

THE CHLAMYDOZOAN HYPOTHESIS OF VON PROWAZEK

The cellular changes associated with virus diseases, particularly the structure and composition of specific cellular inclusions, have been most thoroughly and minutely studied in relation to the aforementioned group of animal poxes. It has been found that there are intracellular inclusions specific for and more or less characteristic of each. Therefore any hypothetic consideration of the nature and significance of the inclusions of virus diseases in general must be based largely on the facts established for variola, vaccinia, sheep-pox, fowl-pox and molluscum contagiosum. There is a certain similarity in the gross lesions and in the characteristics of the inclusions typical of each of these diseases; and in addition to the larger intracellular masses which are designated as the "inclusions" proper, there have been found, by means of smears, stained sections, or by the examination of fresh material, very minute structures, coccoid in form, abundant in number, uniform in size and intracellular. These have been regarded by several investigators as diminutive parasites and the etiologic agents of these diseases. The granules are so small (0.1 to 0.25 micron) that it has not been possible to detect much difference in their morphology in these various infections. It is stated that the bodies are filtrable (da Rocha-Lima), that vaccine inoculum free of them is inactive, and that they may be cultivated in symbiosis with tissue cells (Craciun, Oppenheimer). Morphologic evidence of division has been found in the form of diplococcal and dumb-bell arrangements.

Any plausible theoretical interpretation of the inclusions therefore should include a consideration also of the minute bodies with which they are associated in the diseases mentioned. Three possibilities have been suggested in the literature:

(1) That the inclusions represent stages in the life-cycle of a protozoan parasite, and certain of the minute bodies are of the nature of sporozoites (Calkins).

(2) That the minute bodies are the virus; they invade the cell, injure it, and as a result of its consequent altered activity or actual degeneration the inclusions are formed, from the cellular material (Paschen).

(3) That the minute bodies are the virus which penetrate the cell. The cell responds by forming a plastic material, which collects about the virus, partially or completely mantling it. The resulting product of the cellular reaction together with the incorporated virus constitutes the inclusion (von Prowazek, Lipschütz, da Rocha-Lima).

The third point of view is that on which von Prowazek constructed his hypothesis of *Chlamydozoa,* (mantled organisms), minute, filter-

passing micro-organisms which invade cells and irritate or kill them, thus giving rise, together with various inflammatory phenomena, to the lesions of the disease concerned. On the basis that it is unessential for all viruses of this type to stimulate or injure the invaded cell in such a way that it must react by the formation of an enveloping substance (a mantle), Lipschütz stressed the actual visible form of those minute bodies which he considered to be the real infective agents, rather than the hypothetic cellular response which may or may not be present. He therefore prefers to designate these viruses *Strongyloplasms* (rounded bits of living material).

More recent investigations of the diseases under consideration have not all substantiated the *Chlamydozoa-Strongyloplasm* theory. The observations of Gins on vaccinia (1922) were altogether confirmatory of this hypothesis, while Cowdry (1922), working with vaccinia and employing the method of supravital staining, concluded that the Guarnieri bodies are formed as a result of stimulation of the cells to produce in abundance a material traces of which he found in the same type of cell (corneal epithelium) under normal conditions. Ludford and Findlay (1926) investigating the cellular changes in the lesions of fowl-pox describe the development of Bollinger bodies and consider them to be the result of a reaction of the cell to the virus. They suggest that the bodies may in part be composed of a keratinoid material. Goodpasture (1927) on the other hand in a cytologic study of molluscum contagiosum confirms the observations of Lipschütz, von Prowazek and da Rocha-Lima, as to the presence of the minute components of the inclusions, denominated by Lipschütz, *Strongyloplasma hominis*. Also he finds (1928) in the structure of the inclusions of fowl-pox a most complete substantiation of von Prowazek's hypothesis of *Chlamydozoa,* in that these inclusions are composed of myriads of minute (0.2 micron) bodies suspended in a homogeneous matrix. This matrix may, he thinks, be a product of the micro-organism.

INTRACELLULAR PARASITISM IN VIRUS DISEASES

It seems to be generally accepted that viruses in general are multiplied locally in the specific lesions which they induce, and that there is an intimate relation between the cells affected and the particular virus concerned. The chlamydozoan hypothesis includes the conception of an invasion of cells by virus and implies an active proliferation of minute organisms intracellularly, although it does not exclude an extracellular multiplication. The rupture or destruction of cells would liberate the strongyloplasms which might invade other cells.

Certain investigators, while considering the inclusions to be due to reactive or degenerative changes, seem to believe also that virus actually invades the affected cells and that the alterations observed are due

to the presence of virus intracellularly. Thus Ludford and Findlay, referring to lesions of fowl-pox, state that "the earliest indication of *infection* of an epidermal cell is the formation of a small vacuole"; and that "the disorganization of the cells incident upon keratinization possibly provides conditions for the virus to develop." It appears that the idea of an intracellular parasitism by viruses is more widely applicable to the facts than is the chlamydozoan hypothesis alone.

The suggestion of an intracellular multiplication of viruses has been made on numerous occasions but has not received the attention it deserves. In view of the known facts, it seems to me that a conception of an exclusively intracellular multiplication of virus would be applicable to infections by the etiologic agents of variola, vaccinia, sheep-pox, fowl-pox and molluscum contagiosum, and to those also of herpes and rabies, though morphologic data indicative of a micro-organism as the active agent in the last two is not convincing. In none of these diseases is there evidence of a proliferation of the virus in the blood or body fluids alone. Fowl-pox excepted, virus has not been demonstrated in the blood stream during these infections with any frequency. If virus multiplied in the body fluids independently of cells, it would seem almost certainly cultivatable on nonliving mediums. The filtrable agent of pleuropneumonia of cattle apparently proliferates within the pleural exudate, and no observations have been made which connect this micro-organism intimately with a certain type or group of cells as has been uniformly the case with the other viruses under consideration. The agent of pleuropneumonia is a filter-passing micro-organism which is cultivatable on nonliving mediums (Nocard and Roux).

Many viruses are prone to affect particular types of cells. This fact is emphasized in the use of the expression tropism in reference to certain viruses. Thus the viruses of vaccinia, fowl-pox and molluscum contagiosum are said to be epidermotropic, while those of rabies and poliomyelitis are neurotropic in the same sense. The selectivity of certain viruses for particular cells of the host is one of their most striking attributes, and the idea of an exclusively intracellular growth would be consonant with this important fact.

In this connection it has recently been stated that the quantity of active virus in herpetic lesions of the rabbit's cornea is proportionate to the number of cells containing the characteristic inclusions (Goodpasture), and the suggestion has been offered, with experimental evidence in support of it, that herpes virus is transmitted from a peripheral focus of infection to the central nervous system by its active multiplication within the axis-cylinders of nerves supplying the infected focus. It seems probable also that the viruses of rabies and poliomyelitis may be propagated in the same way, the conception being that these viruses

are essentially intracellular parasites and may find a suitable environment for proliferation within the cytoplasmic processes of nerve cells.

The lesions of the poxlike diseases of animals consist of a hyperplasia of cells to a greater or less degree, together with various degenerative changes. It has been observed that in the lesions of some of them (variola, vaccinia, fowl-pox) mitosis may take place in cells which contain specific inclusions. On the assumption that the inclusions are a manifestation of the local presence of virus it would be logical to assume that such a division would give rise to two infected cells, thus spreading the virus without the necessity of repeated cellular invasion. This is of theoretical interest in connection with those virus diseases which are characterized particularly by cellular proliferation, such as Rous's sarcoma. Lately Borrel described a specific substance situated intracellularly and extracellularly, in Rous's sarcoma. It is suggested that this substance may be formed primarily within the cells and subsequently discharged into the surrounding medium.

SUMMARY

A review of the literature on virus inclusions discloses little uniformity of opinion relative to their composition and significance. The group of animal poxes (variola, vaccinia, sheep-pox, fowl-pox, molluscum contagiosum) presents morphologic evidence, which is lacking or inadequate in practically all other virus diseases, that the infective agent is a minute, rounded, uniform, micro-organism presumably capable of invading cells and of growing intracellularly. According to this conception, in its intracellular situation it induces the changes characteristic of the infection. These changes may be either an irritative hyperplasia, or a swelling, degeneration and necrosis. In molluscum contagiosum and fowl-pox, at least, there is evidence that the cytoplasmic inclusions peculiar to these infections are composed of masses of these small bodies suspended in a hyaline matrix which is either the product of the cell or of the virus itself.

A reviewer of the subject of filtrable viruses cannot but feel regret that the term filtrable has come to be the only term used as definitive of this group of pathogenic agents. He feels it would be of greater value to emphasize other characteristics of such agents which represent their activities and the effects which they induce, rather than to hold tenaciously only to an expression of a single physical characteristic which has become more or less trite. He would like to see some limit placed on the misuse of this group as a repository for all sorts of nondescript and ill defined conditions of unknown etiology and indefinite pathology.

It would seem to be a better practice to exclude from the group of filtrable viruses, or to hold sub judice, those hypothetic agencies

which have not been filtered, which have not been shown to become reproduced at the site of the specific lesion which they cause, and which have not been demonstrated to induce important changes of form or function in the cells on which they exert their specific action and with which they appear to be in some way intimately related.

BIBLIOGRAPHY

Andervont, H. B.: The Relationship of the Epithelioma Contagiosum Virus of Fowls to the Vaccine Virus, Am. J. Hyg. **6**:719, 1926.

Andervont, H. B.: Is the Formation of Guarnieri Bodies an Exclusively Mammalian Response to Infection with the Vaccine Virus? Am. J. Hyg. **6**:618, 1926.

Apolant, H.: Beitrag zur Histologie der Geflügelpocke, Virchow's Arch. f. path. Anat. **174**:86, 1903.

Borrel, A.: Epithelioses infectieuses et epitheliomas, Ann. de l'Inst. Pasteur **17**:81, 1903.

Borrel, A.: Etude experimentale de la clavelée. Filtration du virus, sero-clavélisation; serotherapie, Ann. de l'Inst. Pasteur **17**:123, 1903.

Borrel, A.: Sur les inclusions de l'epithelioma contagieux des oisseaux (molluscum contagiosum) Compt. rend. Soc. de biol. **2**:642, 1904.

Borrel, A.: Cytologie du sarcome de Peyton Rous et substance specifique, Compt. rend. Soc. de biol. **94**:500, 1926.

Bosc, F. J.: Les epitheliomas parasitaires. La clavelée et l'epithelioma claveleux, Centralbl. f. Bakteriol., pt. I, **34**:413, 1903.

Burnet, E.: Contribution a l'etude de l'epithelioma contagieux des oisseaux, Ann. de l'Inst. Pasteur **20**:742, 1906.

Calkins, G. N.: The Life History of Cytoryctes Variolae, Guarnieri, J. M. Research **11**:136, 1904.

Ciuffo: Innesto positivo con filtrato di verruca volgare, Gior. ital. d. mal. ven., ven., Milano, 1907; cited by Lipschütz.

Councilman, W. T.; Magrath, G. B., and Brinkerhoff, W. R.: The Pathological Anatomy and Histology of Variola, J. M. Research **11**:12, 1904.

Cowdry, E. V.: The Supravital Staining of Vaccine Bodies, J. Exper. Med. **36**:667, 1922.

Craciun, E. C., and Oppenheimer, E. H.: The Cultivation of the Granules of Vaccinia Virus, J. Exper. Med. **43**:815, 1926.

Gins, H. A.: Ueber Beziehungen zwischen Tier- und Menschenpocken, Ztschr. f. Hyg. u. Infectionskrankh. **89**:231, 1919; ibid., **95**:255, 1922.

Goodpasture, E. W., and Teague, O.: Experimental Production of Herpetic Lesions in Organs and Tissues of the Rabbit, J. M. Research **44**:121, 1923.

Goodpasture, E. W.: A Cytologic Study of Molluscum Contagiosum, Am. J. Path. **3**:385, 1927.

Goodpasture, E. W.: Certain Factors Determining the Incidence and Severity of Herpetic Encephalitis in Rabbits, Am. J. Path. **1**:47, 1925.

Goodpasture, E. W.: The Pathology of Certain Virus Diseases, Science **67**:591, 1928.

Goodpasture, E. W., on Virus Diseases of Fowls, as Exemplified by Epithelioma Contagiosum of Chickens and Pigeons (Fowl-Pox), in Rivers, T. M.: Filtrable Viruses, Baltimore, Williams and Wilkins Company, 1928, chapt. 7, p. 235.

Guarnieri, G.: Richerche sulla pathogenesi ed etiologia dell' infezione vaccinica e variolosa, Arch. per le sc. med. (Turin and Palermo) **16**:403, 1892; cited by von Wasielewski.

Hartmann: Ueber Chlamydozoen. 4. Tagung der freien Vereinigung für Mikrobiologie in Berlin, 1910, Centralbl. f. Bakteriol., pt. I,, ref. **47**:94, 1910.

Henderson, W.: Notice of the Molluscum Contagiosum, Edinburgh M. & Surg. J. **56**:213, 1841.

Van Heelsbergen, T.: Beitrag zur Kenntnis der Geflügelpocken, insbesondere mit Bezug auf ihre Verwandschaft mit der Vogeldiphtherie, der Stomatitis pustulosa contagiosa equi und der Vakzine, Centralbl. f. Bakteriol., pt. I, **84**: 288, 1920.

Van Heelsbergen, T.: Kuhpocken beim Menschen durch das Virus der Stomatitis pustulosa contagiosa equi. (Der Zusammemhang zwischen der Stomatitis pustulosa contagiosa equi, den spontanen Kuhpocken, den Geflügelpocken und der Vakzine), Centralbl. f. Bakteriol., pt. I, O. **89**:173, 1923.

Hückel, A.: Die Vaccinekorperchen. Nach Untersuchungen an der geimpften Hornhaut des Kaninchens. Ein experimenteller und kritischer Beitrag zur Frage nach den Contagium der Vaccine, Beitr. z. path. Anat. u. z. allg. Pathol., 1898, second supplement, p. 1-130.

Iwanowski, D.: Ueber die Mosaikkrankheit der Tabakpflanze, Centralbl. f. Bakteriol., pt. II, O. **5**:250, 1899.

Juliusberg, M.: Zur Kenntnis des Virus des Molluscum contagiosum des Menschen, Deutsche med. Wchnschr. **31**:1598, 1905.

Lipschütz, B.: Zur Kenntnis des Molluscum contagiosum, Wien. klin. Wchnschr. **20**:253, 1907.

Lipschütz, B.: Weitere Beiträge zur Kenntnis des Molluscum contagiosum, Arch. f. Dermat. u. Syph. **107**:387, 1911.

Lipschütz, B.: Untersuchungen über die Ätiologie der Krankheiten der Herpesgruppe (Herpes zoster, Herpes genitalis, Herpes febrils), Arch. f. Dermat. u. Syph. **136**:428, 1921.

Lipschütz, B.: Filtrierbare Infektionserreger, in Kolle, W., and von Wassermann, A.: Handbuch der pathogenen Mikroorganismen, edition 2, **8**: 345, 1913.

Lipschütz, B.: Untersuchungen über Epithelioma contagiosum der Vogel, Centralbl. f. Bakteriol. **46**:609, 1908.

Lipschütz, B.: Ueber Geflügelpocke, Centralbl. f. Bakteriol. **87**:191, 1921.

Lipschütz, B., Geflügelpocke, in von Prowazek, S.: Handbuch der pathogenen Protozoen **1**:230, 1912.

Lipschütz, B., Filtrierbare Infektionserrege, in Kolle, W., and von Wassermann, A.: Handbuch der pathogenen Mikroorganismen **8**:409, 1913.

Loeffler and Frosch: Berichte der Kommission zur Erforschung der Maul- und Klauensuche bei dem Institut für Infektionskrankheiten in Berlin, Centralbl. f. Bakteriol., pt. I, **23**:371, 1898.

Loewenthal, W.; Kadowaki, Y., and Kondo, S.: Untersuchungen über das Verhältnis der Geflügelpocken zur Vakzine, Centralbl. f. Bakteriol. **94**: 185, 1925.

Loewenthal, W.: Untersuchungen über die sog. Taubenpocke (Epithelioma contagiosum), Deutsche med. Wchnschr. **32**:678, 1906.

Ludford, R. J., and Findlay, G. M.: The Ultramicroscopic Viruses: II. The Cytology of Fowl-pox, Brit. J. Exper. Path. **7**:256, 1926.

MacCallum, W. G., and Oppenheimer, E. H.: Differential Centrifugalization. A Method for the Study of Filtrable Viruses, as Applied to Vaccinia, J. A. M. A. **78**:410 (Feb. 11) 1922.

MacCallum, A. B.: The Histology of Molluscum Contagiosum, J. Cutan. Dis. **10**:93, 1892.

Marx, E., and Sticker, A.: Untersuchungen über das Epithelioma contagiosum des Geflügels, Deutsche med. Wchnschr. **27**:893, 1902.

Marx, E.,and Sticker, A.: Weitere Untersuchungen über mitigation des Epithelioma contagiosum die Geflügels, Deutsche med. Wchnschr. **29**:79, 1903.

Michaelis, L.: Mikroskopische Untersuchungen über die Taubenpocke, Ztschr. f. Krebsforsch. **1**:105, 1904.

Negri, A.: Ueber Filtration des Vaccinevirus, Ztschr. f. Hyg. u. Infektionskrankh. **54**:326, 1906.

Nocard and Roux: Le microbe de la péripneumonie, Ann. de l'Inst. Pasteur **12**:240, 1898.

Paschen, E.: Was wissen wir über den Vakzineerreger? München med. Wchnschr. **53**:2391 (no. 2) 1906.

Paschen, E.: Der Träger des Contagiums der Variola und der Vaccine, Arch. f. Kinderh. **47**:168, 1908.

Polowinkin, P.: Beitrag zur pathologischen Anatomie der Taubenpocke, Arch. f. Thierh. **27**:86, 1901.

Von Prowazek, S.: Handbuch der pathogenen Protozoen **1**:122, 1912.

Von Prowazek, S., and Yamamoto, J.: Experimentelle und morphologische Studien über das Vakzinevirus, München. med. Wchnschr. **56**:2627 (no. 2) 1909.

Von Prowazek, S.: Chlamydozoa. 1. Zusammenfassende Uebersicht, Arch. f. Protistenkunde **10**:336, 1907.

Remlinger and Nouri (Osman): Le virus vaccinal traverse la bougie Berkefeld V. Compt. rend. Soc. de biol. **58**:895, 1905.

Retzius: on Molluscum contagiosum, Deutsch. Klinik, 1871; cited by Lipschütz.

Rivers, T. M.: Filtrable Viruses: A Critical Review, J. Bact. **14**:217, 1927.

Rivers, T. M.: Some General Aspects of Pathological Conditions Caused by Filtrable Viruses, Am. J. Path. **4**:91, 1928.

da Rocha-Lima: Zur Demonstration über Chlamydozoen, Verhandl. d. deutsch. path. Gesellsch. **16**:198, 1913.

Sanfelice, F.: Recherches sur la genèse des corpuscules du Molluscum contagiosum, Ann. de l'Inst. Pasteur **32**:363, 1918.

Serra: Sulla filtrabilite del virus del mollusco contagioso dell' uomo, Boll. d. soc. tra i cult. d. med. e. nat., Gagliari, 1907; cited by Lipschütz.

Toyoda, T.: Versuche über Infektion und Immunität bei verschiedenen Tierpockenarten, Ztschr. f. Hyg. u. Infektionskrankh. **102**:592, 1924.

Tyzzer, E. E.: The Etiology and Pathology of Vaccinia, J. Med. Research **11**: 180, 1904.

Virchow, R.: Ueber Molluscum Contagiosum, Virchow's Arch. f. path. Anat. **33**:144, 1865.

Volpino, G.: Ueber die Beweglichkeit der Körperchen der Vakzine und der Pocken München. med. Wchnschr. **56**:2270 (no. 2) 1909.

von Wasielewski: Beiträge zur Kenntniss des Vaccine-Erregers, Ztschr. f. Hyg. u. Infectionskrankh. **38**:212, 1901.

Wile, U. J., and Kingery, L. B.: The Etiology of Molluscum Contagiosum. Preliminary Report of Experimental Study, J. Cutan. Dis. **37**:431, 1919.

Notes and News

University News, Promotions, Resignations and Appointments.—The following fellowships in medicine have been awarded by the National Research Council: Leon H. Collins, Jr., biochemistry and pathology at the University of Pennsylvania; Herbert L. Ratcliffe, parasitology at the Johns Hopkins University; P. Arthur Delaney, pathology at the University of Chicago; Eugene C. Woodruff, pathology at Vanderbilt University.

Kenneth C. Waddell, assistant professor of pathology, Albany Medical College, has resigned to accept an appointment in the medical service of the Presbyterian Board of Foreign Missions, in Brazil.

At Albany Medical College, Charles M. Carpenter has been appointed research fellow in bacteriology; Ruth M. Boak, instructor in bacteriology and assistant bacteriologist to the Albany Hospital; Yvonne de la Pasture, instructor in pathology and resident pathologist to the Albany Hospital, and Charles E. Martin, assistant in pathology.

George J. Rukstinat, Eustace L. Benjamin, Samual A. Levinson, Chester C. Guy, Francis D. Gunn, Hamilton R. Fishback and Paul G. F. Schmitt have been appointed coroner's physicians of Cook County (Chicago) by Dr. Herman N. Bundesen, the first physician to be coroner in Cook County in sixty-five years.

Thomas B. Haslan has been appointed associate professor of pathology at Baylor University, Dallas, Texas, and medical director of the Baylor Hospital Clinic.

At Stanford University, Claus W. Jungeblut has been promoted from assistant professor of bacteriology to associate professor for five years.

It is reported that Werner Gerlach, Hamburg, has been appointed professor of pathology in the University of Halle.

At the University of Kansas, Raymond F. Gard has been appointed instructor in pathology and resident pathologist to the Bell Memorial Hospital.

At the Harvard Medical School, John Reginald Cuff has been appointed research fellow in pathology and John Archibald Ferguson instructor in pathology.

Ella Sachs Plotz Foundation.—According to the fifth annual report, twenty-one grants, totaling approximately $12,000, were made during 1928. Twelve of these grants were made to scientists in countries outside the United States. In making grants, the trustees of the foundation favor researches directed toward the solution of problems in medicine and surgery or in branches of science bearing on medicine and surgery. Grants may be used for the purchase of apparatus and supplies needed for special investigations, and for the payment of unusual expenses incident to such investigations, including technical assistance, but not for providing apparatus or materials which ordinarily are part of laboratory equipment. Stipends for the support of investigators are granted only under exceptional circumstances. Applications for grants should be made before May 15, 1929, to Dr. Joseph C. Aub, 695 Huntington Ave., Boston.

Fund for the Study of Influenza.—The Metropolitan Life Insurance Company has appropriated $10,000.00 for the study of influenza by the company's influenza commission, of which M. J. Rosenau is chairman.

Archives of Malignant Diseases.—The American College of Surgeons, Chicago, has appointed a committee to collect statistics bearing on the relation of heredity to human neoplasms. Dr. Bowman C. Crowell, 40 East Erie Street, Chicago, is the chairman of the committee.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

DIABETES AND HYPERTHYROIDISM. ELLIOT P. JOSLIN and FRANK H. LAHEY, Am. J. M. Sc. **176**:1, 1928.

The analogy between diabetes and hyperthyroidism is emphasized and its importance in the interpretation of the symptoms, in the course of the disease and in the treatment is discussed. The patient with hyperthyroidism, from physiologic, pathologic and statistical evidence, is somewhat more prone to diabetes than other persons, and for the remainder of his life should be so regarded, whether operated on or not.

PEARL ZEEK.

CARDIOVASCULAR FINDINGS IN WOMEN WITH SYPHILIS. JOHN H. ARNETT, Am. J. M. Sc. **176**:65, 1928.

Two hundred and five female dispensary patients with tertiary syphilis, twenty-five with secondary syphilis and seventy-eight without this disease, as controls were subjected to a uniform examination, including, whenever possible, an electrocardiogram and an orthodiagram. Evidence was not found for the belief that, in the absence of definite evidences of cardiac impairment, syphilis by itself may produce cardiac enlargement. In 3.2 per cent of the tertiary group, a diagnosis of aortitis was made and in 2 per cent a diagnosis of aortic regurgitation. Arterial hypertension was slightly more common in the tertiary group, and arterial hypotension in the secondary group, than in the controls. T-wave deflects were frequently noted in the tertiary group. Tachycardia was more common in both syphilitic groups, than in the controls. Organic cardiovascular disease was not demonstrated in any of the patients with secondary syphilis.

PEARL ZEEK.

NITROGEN ELIMINATION AND THE RESIDUAL NITROGEN FRACTION IN THE URINE. ALLAN WINTER ROWE and BERNARD EMERSON PROCTOR, Am. J. M. Sc. **176**:103, 1928.

In certain states of disease, that portion of the urinary nitrogen which remains after the urea, uric acid, ammonia and creatinine fractions have been deducted from the total definitely exceeds the limits characteristic of normal urine. In a series of cases including both normal persons, as controls, and patients with a variety of diseases, the amino-acid, hippuric acid and creatinine fractions were determined. While in cases showing high residuals there were increases in these constituents, the total left an undetermined fraction to be accounted for. Patients in whom the gonad and thyroid glands failed to function showed large increases in the creatinine output.

PEARL ZEEK.

NITROGEN ELIMINATION AND THE SULPHUR PARTITION. ALLAN WINTER ROWE and BERNARD EMERSON PROCTOR, Am. J. M. Sc. **176**:114, 1928.

Further study of the nature of the residual nitrogen fraction in the urine reveals an increase in the neutral sulphur content in certain pathologic conditions above the level shown by the controls, but this increase is not commensurate with the observed increase in the undetermined nitrogen portion of the same urine. Therefore, it seems evident that in cases of high residual nitrogen fractions, the increase is due not only to a larger percentage of the known materials usually present in small amounts, but also to the appearance of some other nitrogen-containing material of unknown origin and composition.

PEARL ZEEK.

THE CAPILLARY PRESSURE IN THE TOXEMIA OF PREGNANCY. ISADORE MUFSON, Am. J. Obst. & Gynec. **15**:800, 1928.

Mufson shows that there is not any characteristic morphologic picture to be found in the capillary bed corresponding with the normal or the clinical hypertension of pregnancy. The capillary pressure is, however, shown to be a definite prognostic indicator; when high, it proves to be unfavorable to mother and fetus. The capillary pressure following intravenous injection of magnesium sulphate shows a prompt reduction simultaneous with that of the brachial pressure, but the decrease is followed by a gradual return to the original reading.

A. J. KOBAK.

INCREASE IN SUGAR METABOLISM BY THE OVARIAN HORMONE. A. M. ESTES and W. E. BURGE, Am. J. Obst. & Gynec. **15**:847, 1928.

The results reported are based on the rate of sugar absorption by paramecia as influenced by the addition of graded amounts of ovarian hormone. Washed paramecia were introduced into 500 cc. of aerated lake water containing 500 mg. of dextrose, and after thorough mixing this preparation was equally apportioned among five tubes. One tube was kept as a control and the rest received 25, 75, 100 and 200 mg. of ovarian hormone, respectively. The sugar content was analyzed twelve hours later, and found to be more and more decreased with the greater amount of hormone up to the amount of 200 mg.; with this amount it was found slightly decreased. This was assumed to have been due to a depressive action on the part of the large dose of hormone.

A. J. KOBAK.

TOXICITY OF INTESTINAL CONTENTS IN HEALTH AND IN INTESTINAL OBSTRUCTION. O. H. WANGENSTEEN and STANLEY S. CHUNN, Arch. Surg. **16**:606, 1928.

The content of the small intestines of several normal dogs and rabbits was injected intravenously, after Berkefeld filtration, into normal dogs and into the peritoneal cavity of rats. Also the intestinal content taken from above and from below the level of intestinal obstruction was similarly injected into normal animals. Of seventeen dogs thus receiving injections, two died, and in both of these the material injected was from the intestines of normal animals. All the dogs were ill with listlessness, vomiting, diarrhea, salivation, stupor and coma. Of thirty-six rats receiving the injections, eleven died. Nine of these had received injections of intestinal contents from below the point of obstruction. One died as a result of the injection of the normal content, and one as the result of the injection of material obtained from above the point of obstruction. Fifteen rats received injections of the intestinal content of normal rabbits, and five died. Of fifteen rats receiving injections of the content of the obstructed bowel, three died. When the material was not filtered through the Berkefeld filter, it caused death in a great percentage of the animals. From their experiments, the authors conclude that the normal intestinal content of both the dog and the rabbit is toxic on injection when submitted to the same treatment as the content of obstructed intestines. The material obtained from below the point of obstruction in dogs was even more toxic than the material obtained proximal to the obstruction.

N. ENZER.

CONTRACTION OF THE GALLBLADDER IN THE COMMON BULLHEAD (AMEIURUS NEBULOSIS). G. M. HIGGINS, Arch. Surg. **16**:1021, 1928.

With proper aerating of the fish, it was possible to open the animal and observe the biliary tract and its response to the intraduodenal injection of cream and egg yolk. This injection elicited almost at once an active gastro-intestinal peristalsis and antiperistalsis. After about one hour and fifteen minutes, increased tonus in the gallbladder was apparent; this was followed by waves of contraction that emptied the contents into the common duct. The evidence points to an active

contraction of the muscle in the wall of the gallbladder, the degree of contraction varying according to the gastro-intestinal absorption of fat. Duodenal peristalsis and intra-abdominal pressure are not factors in the discharge of bile from the gallbladder.

N. ENZER.

THE METABOLISM OF LIVER TISSUE FROM RATS OF DIFFERENT AGES. J. A. HAWKINS, J. General Physiol. **11**:645, 1928.

A study of the respiration, aerobic glycolysis and anaerobic glycolysis of the livers of rats of various ages did not show differences for the first two processes. In the livers of old and normal adult rats there was practically no anaerobic glycolytic activity, but there was some in the livers of very young rats. This accords with the writer's view that the glycolytic activity of a tissue is a function of its rate of growth.

H. E. EGGERS.

CHANGES IN BLOOD DEXTROSE IN RABBITS AFTER INTRAVENOUS INJECTIONS OF HISTAMINE. MAUD L. MENTEN and HELEN M. KRUGH, J. Infect. Dis. **43**:117, 1928.

The convulsions and anaphylactic shock produced by lethal doses of histamine in the rabbit may be accompanied either by no appreciable change in the blood dextrose or by a maximal hyperglycemia.

The experiments reported indicate that allergy is not a factor in the production of agonal hyperglycemia.

AUTHORS' SUMMARY.

INSULIN OF THE PANCREAS FOLLOWING INTOXICATION OF RABBITS WITH PARATYPHOID B FILTRATE AND DYSENTERY BACILLI. MAUD L. MENTEN and HELEN M. KRUGH, J. Infect. Dis. **43**:121, 1928.

Single intravenous sublethal injections of paratyphoid B filtrates do not appreciably alter the insulin content of the pancreas, as demonstrated by comparative biologic assays in test rabbits.

The hypoglycemic potency of the insulin extract of the pancreas from rabbits in which injections of *B. dysenteriae* (Shiga) had caused a prolonged fatal toxemia and hyperglycemia was slightly diminished.

AUTHORS' SUMMARY.

THE ASSOCIATION OF BOWEL DISEASE WITH VITAMIN C DEFICIENCY. F. P. MACKIE and G. D. CHITRE, Indian J. M. Research **16**:77, 1928.

Monkeys on a diet deficient in or lacking vitamin C become debilitated and anemic, lose weight rapidly and generally suffer from a terminal fatal dysentery. Definite signs of scurvy appear in most of the animals. This scorbutic condition can be checked by the administration of orange juice or by a return to a normal dietary, provided it is not advanced. When once the scorbutic condition is well established, this treatment cannot be depended on to save the animals' lives. The postmortem signs are most marked in the large intestine, which shows a succession of changes suggestive of local poisoning. These signs vary from local congestion and thickening of the mucous membrane to a condition indistinguishable from ulcerating and sloughing dysentery. The morbid histology suggests degenerative changes due to the action of a toxin, to which inflammatory changes are added secondarily. Specific excitants of dysentery such as amebas or dysentery bacilli are not found in the large majority of cases. The commoner fecal flora, in some cases, are found to have invaded the living tissues of the bowel wall, and this suggests that the toxic changes observed are the effect of such bacteria acting on tissues devitalized by the ill-balanced dietary. These changes appeared in the alimentary canal in all animals kept on a diet deficient in vitamin C whether given infective sprue material or not. Monilia were not found to have invaded the diseased areas, nor can the changes be attributed to their action. A defective

dietary by itself powerfully predisposes to bowel derangements and acts probably by reducing the natural resistance of the intestinal epithelium to the invasion of bacteria or their toxins.

AUTHORS' SUMMARY.

STUDIES ON THE PHYSIOLOGY AND PATHOLOGY OF THE GASEOUS EXCHANGE IN THE TUBERCULOUS. F. POMPLUN, Ztschr. f. Tuberk. **50**:387, 1928.

The color of the blood is not an indication of the amount of oxygen absorbed. The oxygen values of the venous blood of patients with far advanced tuberculosis are decreased. This decrease is parallel with this calculated relative deficit of oxygen. The blood of patients with far advanced tuberculosis shows higher carbon dioxide values than the blood of those with less advanced tuberculosis. Low oxygen values are frequently found in the presence of high carbon dioxide values. Patients with artificial pneumothorax do not show any deviation from the normal. Analyses of arterial blood yielded essentially similar results.

MAX PINNER.

INFLUENCE OF THE THYROID ON THE REGENERATION OF CORNEAL EPITHELIUM. M. MINOWADA, Acta Dermat. **11**:437, 1928.

In thyroidectomized animals, epithelialization of the surface of the cornea is delayed as compared with the process in controls treated with thyroid substance.

DIFFERENTIAL DIAGNOSIS OF GLYCOSURIAS. Y. ÅKERRÉN, Upsala Läkare. Förh. **34**:341, 1928.

In seven healthy or convalescent subjects, three patients with glycosuria who showed normal fasting blood sugar value from the start, and two patients with diabetes with remission, Åkerrén found the fasting blood sugar value normal during and after a special diet consisting of the usual mixed diet with at least 400 Gm. of bread and at least 100 Gm. of sugar daily (the sugar in the usual food not included). He says that examination along these lines may be considered a function test of the carbohydrate metabolism of the organism. A patient with glycosuria who maintains a normal fasting blood sugar value under this forced carbohydrate intake shows complete carbohydrate tolerance and may be regarded essentially as a normal organism with respect to carbohydrate metabolism.

Pathologic Anatomy

SPONTANEOUS HEART RUPTURE. A. B. DAVENPORT, Am. J. M. Sc. **176**:62, 1928.

Statistical data are given in reports of ninety-two cases of heart rupture collected from the literature and the following conclusions drawn: Spontaneous rupture of the heart is a disease of the aged, occurs most often on the anterior surface of the left ventricle and, in the aged, is practically always the result of coronary disease with infarction.

PEARL ZEEK.

FREQUENCY AND DISTRIBUTION OF FOSSA PHARYNGEA IN HUMAN CRANIA. HENRY B. COLLINS, JR., Am. J. Phys. Anthrop **11**:101, 1927.

Human craniums to the number of 9,135 in the U. S. National Museum were examined, and the frequency of the fossa pharyngea noted. As to sex, the fossae were distributed as follows: females, 6.55 per cent; males, 4.98 per cent; children, both sexes, 5.35 per cent. The Melanesians show the highest frequency of the

fossa; the white races, the lowest. Among American Indians the highest frequency is found among the Shoshonean-speaking tribes, followed closely by the Sioux, Apache, and other Plains tribes, and the Aleutians. The Eskimos show the lowest frequency of any American group.

AUTHOR'S ABSTRACT.

SEX DIFFERENCES IN THE PATHOLOGIC PICTURE OF SYPHILIS. ALDRED SCOTT WARTHIN, Am. J. Syph. **12**:301, 1928.

In a summary of the differences in the pathologic changes of syphilis in the two sexes, the most striking facts presented are those bearing on the relatively greater immunity of the female to this infection than the male possesses. This immunity is shown particularly in the case of the heart, aorta, central nervous system and ovary. On the other hand, the pathologic lesions of latent syphilis in women are usually more extensive in the liver, pancreas, suprarenals and rectum than in men, but severe clinical forms of these visceral lesions are relatively infrequent when compared to the severe forms of cardiovascular syphilis and that of the central nervous system in man. The general aspects of syphilis in the female, therefore, tend to be much milder than in the male. Woman's relatively greater immunity to syphilis is most strikingly shown during the child-bearing period. During this time she may not have any clinical symptoms of syphilis, and the Wassermann reaction may be negative. Nevertheless, such an apparently normal woman may bring syphilitic children into the world, and the placenta, umbilical cord and fetal tissues of her progeny may show spirochetes in enormous numbers. The production of a syphilitic child may be the only diagnostic fact that can be determined. Not until the menopause approaches may signs of syphilitic lesions and a positive Wassermann reaction appear. It is this aspect of syphilis that makes the problem of this disease of such tremendous sociologic importance. Many reasons have been advanced in explanation of this immunity in women: differences in the character of the serums in the two sexes, that of women having greater protective power against the spirochetes; greater degree of lymphocytosis, protective action of the thyroid and other endocrinal secretions in women; differences in habits of the two sexes; conceptional protection through chorionic proteins, etc. The facts stand out, however, that in addition to a general modification of the disease as affecting the body as a whole, there is also in the woman a marked localized resistance to syphilis in certain organs and tissues. It is difficult to explain the exemption of the ovary in any other way, when it is known that the male sex gland has an especial susceptibility to syphilitic injury. It does not seem possible that evidences of syphilis in the ovary is being missed; it is much more reasonable to believe that syphilitic lesions do not occur in the ovary. If they do, they must be totally different from those seen in other organs. All in all, one can only surmise that there is some deep-seated biologic sex difference—a genetic, inherited, sex-limited resistance on the part of the ovary to the presence of the spirochete. It might be that *Spirochaeta pallida* is a pathogenic descendant of some harmless spirochetal form inhabiting the female body ages ago, and that in consequence woman establishes a more comfortable partnership with this organism than does man. Such a theory does not, however, explain a higher degree of immunity of the woman during the period of child-bearing, and the apparent increase of immunity as the result of conceptions. Whatever may be the explanation of woman's relative immunity to this infection, whether one or several of these factors contribute to it, the important fact remains that syphilis manifests itself in the female almost as if she were another animal species.

In regard to this sex-difference in reaction to infection with *Spirochaeta pallida*, there center not only grave sociologic problems but also the most diffcult practical problems in the diagnosis and treatment of syphilis. AUTHOR'S SUMMARY.

MASSIVE COLLAPSE (ATELECTASIS) ASSOCIATED WITH PULMONARY TUBERCULOSIS AND TUMOR. EDWARD N. PACKARD, Am. Rev. Tuberc. **18**:7, 1928.

Five cases are cited which exhibited the signs and symptoms of massive collapse, but in none of these did an operation precede the development of the atelectasis. Four of these patients had chronic pulmonary tuberculosis. One case was complicated by carcinoma, and one patient had a tumor of the lung. Autopsies in two of the cases showed that the collapse of lung tissue was due to bronchial obstruction. Massive collapse of the right lung may be one of the causes of acquired dextrocardia. Rapid, massive pulmonary fibrosis of a lobe or of the whole lung is probably etiologically a massive atelectasis. H. J. CORPER.

NODULAR GOITER WITH HYPERTHYROIDISM. H. M. THOMAS, Arch. Surg. **16**: 117, 1928.

In thirty-two cases of nodular goiter presenting clinical symptoms of hyperthyroidism, sections revealed areas of hypertrophy and hyperplasia. A close parallelism can be traced between the degree of morphologic change and the severity of the symptoms. In this series of nodular goiters, the only difference between them and true exophthalmic goiter seems to be one of degree. The conclusions fit in with Rienhoff's hypothesis. N. ENZER.

OBSTRUCTIVE MASSIVE ATELECTASIS OF THE LUNG. P. N. CORYLLOS and G. L. BIRNBAUM, Arch. Surg. **16**:501, 1928.

The authors offer this definition of the condition: "Obstructive massive atelectasis of the lung is a febrile complication which appears within a few days after operation, generally on the abdomen, and is always due to a more or less temporary but complete obstruction of the bronchus and followed by the more or less complete absorption of vesicular air in the corresponding portion of the lung, thus giving the organ the structure of a fetal lung. It is especially and often conclusively characterized by the clinical symptom of a unilateral, pulmonary consolidation with displacement of the mediastinum and the heart toward the affected side. The prognosis is generally favorable, and ends in lysis or crisis. The treatment is bronchoscopic aspiration of the obstructing mucus as early as possible." N. ENZER.

ADENOMYOMA OF THE STOMACH. J. H. WOOLSEY and R. J. MILLZNER, Arch. Surg. **16**:583, 1928.

At operation for what was suspected to be a jejunal ulcer, a small ringlike thickening was found on the anterior surface of the pylorus. This was excised and examined. Grossly the mucosa was normal over the area of thickening, and the transverse section through it gave the picture of an old callous ulcer. Microscopic examination revealed intact, essentially normal mucosa, a thick, fibrous submucosa, and adenoma scattered throughout the submucosa and muscularis. The glands were of three types—more or less undifferentiated ones, glands resem-

bling pancreatic acini, and others resembling Brunner's glands. Between the glands were narrow strands of smooth muscle which more or less surrounded the alveoli.

The condition of adenomyoma of the stomach is undoubtedly a heterotopia developing from epithelial buds or diverticula, the commonest form of which is an accessory pancreas. One of the interesting statements in the article is that the alveoli open into ductlike structures, which eventually communicate with the lumen of the stomach. This is interesting because the reviewer made serial sections on two accessory pancreases found in the wall of the ileum, and was unable to find any communication with the lumen, although there were several distinct duct structures in the nodules. So far, the malignant degeneration of the tumors has not been described except in reports of three cases of accessory pancreas.

N. ENZER.

BLOOD IN THE CEREBROSPINAL FLUID; CLINICAL DATA. C. BAGLEY, Arch. Surg. **17**:39, 1928.

The most interesting and important part of this paper lies in the report of twenty-seven cases in which bloody cerebrospinal fluid was present with various types of underlying lesions. Five of these patients had cerebral aneurysms. In the majority, the hemorrhage was due to trauma. The presence of blood in the spinal fluid constitutes a meningeal irritant, and the symptoms depend on the degree of hemorrhage. These may range from slight headache to severe convulsive seizures and loss of consciousness. The symptoms may subside as the blood is absorbed, but the irritation may be sufficiently severe to cause a connective tissue proliferation and blockage of the foramina.

N. ENZER.

FAT EMBOLISM; INCIDENCE AT POST MORTEM. E. P. LEHMAN and R. F. MCNATTIN, Arch. Surg. **17**:179, 1928.

In a previous article (ARCH. SURG. **14**:621, 1927) the authors concluded that the fat found as embolism in nontraumatic cases probably appeared by the coalescence of the ultramicroscopic particles of fat normally appearing in the blood plasma. This coalescence was attributed to a breaking down of the physiologic emulsion from physical or chemical changes in the blood, and was produced experimentally in the living dog by the administration of ether. Because of the generally accepted observation that if fat embolism occurs it will be seen in the lung, the lungs of fifty unselected patients were examined for fat. Intracapillary globules of fat were diagnosed as fat embolism. Most of these were large enough to plug capillaries, and the degree of embolism was graded according to the amount found in a section. It seems possible that a source of free fat in the blood stream may be the distribution of fat-containing leukocytes. With one exception, wherever leukocytes were filled with fat, embolism also occurred. Definite fat embolism occurs in more than half of all lungs examined at autopsy. About half of the lungs in which fat embolism occurs are from patients in whom there has been no history of trauma. In a small proportion of cases in which definite trauma is recorded, emboli could not be found on a single examination. Fat embolism is found in an overwhelming proportion of lungs from patients subject to injury or operation. Fat embolism occurs in more than half of the miscellaneous diseases without history or trauma. The presence of fat embolism in the lung after trauma does not justify the conclusion that such fat has been caused by the trauma, or that it has caused death.

N. ENZER.

ARTERIOVENOUS ANEURYSM OF THE BRAIN. W. E. DANDY, Arch. Surg. **17**: 190, 1928.

Eight arteriovenous enurysms of the brain are presented, and twenty-two cases from the literature. Arteriovenous aneurysms of the brain are similar to those elsewhere in the body, except that traumatic arteriomenous aneurysms probably do not occur in the brain, because large arterial and venous trunks are not in apposition. There are two of these types of arteriovenous aneurysms, one in which an anomalous vessel of congenital origin establishes a direct end-to-end communication between an artery and vein, the other in which a network of vessels, a so-called angioma, is interposed between an artery and one or several veins. A capillary bed between the artery and vein is lacking in both types; the arterial blood passes directly into the veins. They constitute 1 per cent of a series of tumors of the brain. Hemorrhage was present in 40 per cent of these aneurysms.

N. ENZER.

ASCARIASIS OF THE GALLBLADDER. CHARLES BRUCE MORTON, Arch. Surg. **17**:324, 1928.

The literature was searched for reports of cases of ascariasis of the gallbladder. Seven cases were found, and five have been briefly summarized. A case of teniasis of the gallbladder was found and also summarized. It was similar in many respects to the cases of ascariasis. All cases had been diagnosed only after operation or necropsy. A case of ascariasis of the gallbladder which I encountered has been described, and the condition discussed in relation to its etiology, diagnosis and treatment. It was pointed out that the disease is rare and difficult to diagnose. Furthermore, the treatment is surgical, and cholecystectomy is probably the operation of choice. Anthelmintics are indicated for prophylactic and postoperative use. The history of ascariasis in a patient with signs of cholecystic disease should always suggest the possibility of ascariasis of the gallbladder.

AUTHOR'S SUMMARY.

SIMPLE, NONSPECIFIC ULCER OF THE COLON. M. E. BARRON, Arch. Surg. **17**:355, 1928.

Those ulcers not due to any known specific cause are considered in this article. Fifty cases collected from the literature are reported in detail, and the author adds three cases from his own experience. The ulcers may be single or multiple, and may occur anywhere in the colon. Thirty-one of these ulcers were in the right portion of the colon. In this series, they were much more common in males. The diagnosis may be suspected from a history of persistent localized pain and with the aid of the x-rays. This may show a perforating defect or an encroachment on the lumen by a mass. This mass is inflammatory and must be carefully distinguished from malignant disease. The diagnosis is usually made after perforation when an exploratory operation is performed. Barron thinks that these ulcers should be classed with the peptic ulcers and are related to them by a common etiology.

N. ENZER.

"STREAM LINE" PHENOMENA IN THE PORTAL VEIN AND THE SELECTIVE DISTRIBUTION OF PORTAL BLOOD IN THE LIVER. G. H. COPHER and B. M. DICK, Arch. Surg. **17**:408, 1928.

By injecting trypan blue into the tributaries of the portal vein of dogs, the authors demonstrate that the site of its deposit in the liver will depend on the

vessels into which injection is made. Thus when injections of the dye were made into a vein on the lesser curvature of the stomach or into a branch of the splenic vein, it stained only the left half of the liver. The veins of the upper part of the duodenum, head of the pancreas and jejunum carried the dye to the right lateral lobes. Those from the colon distributed the dye to all parts of the liver, but more particularly to the large lobe on the left. By a process of transillumination of the portal vein, the authors were able to demonstrate that three streams of blood are present in that vein, and that they correspond to the drainage from the three regions previously mentioned. It would seem, therefore, that certain areas of the liver are supplied by blood from fairly well-defined intra-abdominal regions and that the blood from one region does not mingle with that from another either in the portal vein or in the liver.

N. ENZER.

CARCINOMA OF THE ISLANDS OF THE PANCREAS: HYPERINSULINISM AND HYPOGLYCEMIA. WILLIAM THALHIMER and FRANCIS D. MURPHY, J. A. M. A. **91**:89, 1928.

After two and a half years of gradually increasing severity of symptoms, the patient developed a state of almost constant semistupor accompanied by frequent convulsions and marked hypoglycemia until death followed, apparently from exhaustion. At necropsy the only significant pathologic lesion found was a small primary tumor of the pancreas. Its microscopic appearance indicates that it originated from the cells of the islands of Langerhans, and that the tumor is either a carcinoma of a low grade of malignancy or else an adenoma. It is believed that the active secretion of insulin by these tumor cells caused a hyperinsulinism which resulted in hypoglycemia and, finally, in death.

AUTHORS' SUMMARY.

RECOVERY OF HUMAN OVA FROM UTERINE TUBES. EDGAR ALLEN, J. P. PRATT, O. U. NEWALL and LELAND BLAND, J. A. M. A. **91**:1018, 1928.

This report records the recovery of seven ova from the uterine tubes, among them one set of twins and one case of internal migration of an ovum from one ovary to the other tube. One ovum was recovered on the twelfth, four (including the twins) were recovered on the fifteenth and two were recovered on the sixteenth day of the menstrual cycle. The last two showed signs of degeneration, and further correlation of their condition with the histology of the corresponding corpora lutea may place their time of ovulation earlier in the cycle.

AUTHORS' SUMMARY.

THE PATHOLOGY OF THE MEMBRANES OF BRAIN AND SPINAL CORD. THOR ROTHSTEIN, L. W. AVERY and R. RICHTER, J. Infect. Dis. **43**:1, 1928.

The cells of the dura and of the pia-arachnoid form a syncytial membrane spinning through the whole thickness of the respective meninges, which demonstrates that the original mesenchymal syncytium remains permanent through life, so far as dura and pia are concerned. The so-called "membranes" of the meninges, instead of being only supporting structures, are protoplasm having the constituents for highly important functions.

The so-called surface endothelium forms an integral part of the syncytium and has fibroblastic characteristics.

Reticulum consist of protoplasm with cytoplasmic fibrillae. In the protoplasm which contains or accompanies the reticular fibers, mitochondria can be demonstrated. Fibroglia is formed from the cytoplasmic fibrillae, and collagenic fibers also arise from cytoplasmic fibrillae.

AUTHORS' SUMMARY.

STUDIES ON SCURVY. ARTHUR W. MEYER and LEWIS MCCORMICK, Stanford University Press, 1928, pp. 1071.

THE SYMPTOMATOLOGY AND GROSS MORPHOLOGY OF EXPERIMENTAL SCURVY IN THE GUINEA-PIG. ARTHUR W. MEYER.

As far as the gross lesions regarded as diagnostic of experimental scurvy in the guinea-pig are concerned, the author adds little except that the presence of fatty degeneration in the liver and absorption on the roots of the molar teeth, with the possibility of depressing the posterior or all of the molars, are constant morphologic signs. The extent of depression that is possible always is greatest in the last molars and decreases from back forward. Outwardly evident lesions in the bones and joints usually are absent in the mature guinea-pig, and such animals may show evident signs of pain without the presence of swollen or tender joints and without hemorrhage in the extremities. They also may be active and apparently unaffected and yet have extensive hemorrhages and may have suffered considerable absorption on the roots of the molars and in the alveoli. Some subcutaneous and muscular hemorrhage is almost always present somewhere at the time of autopsy, and duodenal, cecal and vesical (urinary) and paravesical hemorrhages are common. The stomach and, in fact, the entire intestinal canal, usually contains little food, and I have come to regard the lack of appetite and interest in food and also the lessened activity among the earliest signs of the existence of the scorbutic condition. An entire lack of interest in food always was an absolute indication of death within a few days no matter how well the animal seemed or how active it was. Increasing inactivity and weakness and increased loss in weight and appetite usually enabled one to forecast the approximate time of death except for the advent of sudden complications. The new observations on the gross pathologic condition of experimental scurvy in guinea-pigs here reported are: the highly nervous condition of some of the animals; the recovery of health of young pigs after a condition of complete helplessness in the posterior half of the body; the occurrence of permanent locomotor disabilities; shortening of the roots of the teeth by absorption so that the molars can be depressed below the level of the gums; the occurrence of pronounced gaseous distention of the stomach in some animals, and pseudopneumonic lesions in the lungs of others; fatty change, even to whiteness, especially of the liver, to a lesser degree of the suprarenals, and microscopically also in other organs, as will appear in part II; necrotic areas in both liver and suprarenals; degenerative changes in and fibrosis of the costal cartilages even in regions remote from the costochondral junctions.

THE MINUTE MORPHOLOGY OF EXPERIMENTAL SCURVY IN THE GUINEA-PIG. ARTHUR W. MEYER.

Hemorrhages were noted rarely in the skin, almost universally somewhere in the subcutaneous tissues, commonly in the muscles, the urinary bladder, the periostea, the periodontia, many glandular organs, and the lungs, stomach and intestine. They were also seen in the gallbladder, the brain, spinal cord, posterior root ganglions and the nerve trunks. Intragingival and intra-articular hemorrhages were not observed. The marked fatty infiltration and degeneration evident grossly in the liver and sometimes also in the kidney were found present microscopically in the kidney, the suprarenal, and even in the lung and the peribronchial cartilages, the pancreas, some skeletal muscles, and the walls of a blood vessel. Gegenerative changes other than fatty changes, resulting in the complete loss of substance, were observed in the cartilages, bones, teeth, muscles, many glandular organs and also in the central, the peripheral and the sympathetic nervous systems. This widely distributed liquefaction of the cytoplasm and cell walls results in destruction of cartilage cells and of the cartilaginous matrix, at least in the costal cartilages; in the detachment of the periostea and periodontia; in reduction in the caliber of the bones, and in both the caliber and the length of the implanted portions of the teeth. It may effect not only the separation of ununited epiphyses and loosening of the teeth, but the complete destruction of the parenchyma of some areas in the glandular and other organs, in desquamation of the mucosa

and of renal epithelium, and in complete disintegration of the walls of blood vessels. In addition to the fatty and lytic changes, coagulative changes, such as extreme waxy degeneration in the muscles, were also noted in glandular and nervous tissues. The only proliferative changes noted occurred in the costal cartilages and concerned an increase in caliber at the region of the costochondral junctions and an invasion of connective tissue into areas of degeneration. Necrotic areas on the surface and in the substance of the liver were occasionally observed, but perhaps were wholly unrelated to scurvy. Vacuolation was common in many organs, and fenestration up to a marked degree was observed, especially in muscle, bone marrow, the pulp of the teeth, and also in the cord and brain.

SOME CHARACTERISTICS OF THE BLOOD OF THE GUINEA-PIG IN EXPERIMENTAL SCURVY. LEWIS M. MCCORMICK.

Definite cytological changes occurred in the blood of guinea-pigs with experimental scurvy. These changes became evident after the pigs were on the author's scorbutic diet for ten days. They consist of a decrease in red blood cells, hemoglobin and color index, an "apparent" decrease in fragility, a relative decrease in the number of lymphocytes, an absolute increase in polymorphonuclears and an increase in reticulated and nucleated red blood cells and leukocytes. The eosinophil, basophil, monocyte and transitional cell counts were not considered characteristic of scurvy.

AUTHORS' SUMMARY.

CONGENITAL ANEURYSM OF THE CEREBRAL ARTERIES. F. GREEN, Quart. J. Med. **21**:419, 1928.

From 1922 to 1927 at St. Bartholomew's Hospital, London, nineteen bodies at autopsy presented basal cerebral aneurysms, and in seventeen death was due to their rupture. Five of these were embolic and associated with malignant endocarditis; two were associated with extensive arteriosclerosis, and twelve were congenital. In three bodies two aneurysms were found, and in one three saccular dilatations were seen on various branches of the circle of Willis. The average age of death in the embolic type was 25 years, in the sclerotic type, 58 and in the congenital type, 43. The average age at death from lenticulostriate and pontile hemorrhage was 58 years. The three reports under consideration concerned: a single woman, aged 30, who lived for six weeks with leakage from a saccular aneurysm of the right middle cerebral artery; a married woman, aged 49, who died two days after the onset of bleeding from an aneurysm identical with the foregoing one, and a man, aged 21, who had suffered repeated attacks of headache and loss of consciousness. Death occurred in the last patient fifteen hours after the last attack, and at autopsy three aneurysms were found associated with coarctation of the aortic isthmus, a bicuspid aortic valve and fenestrated mitral cusps. Histologically, the essential changes in the aneurysm walls were atrophy and connective tissue replacement of the media occurring frequently at a single point in the circumference and associated with splitting of the elastic lamina and subendothelial hyperplasia of the intima.

GEORGE RUKSTINAT.

LIPOMA OF CEREBRIFORM TYPE. A. BOTTO-MICCA, Gazz. d. Osp. **49**:451, 1928.

A rare type of lipoma in a married woman, aged 25, is reported by Botto-Micca. The case is interesting chiefly from the clinical side. The tumor, which was located on the back between the twelfth dorsal and the third lumbar vertebrae, consisted of a roundish yellow mass, about 10 cm. long, 7 cm. wide, and 1 cm. thick, with an oily serous excretion. When first discovered by the mother, when the daughter was 14, it had about the same size and appearance as shortly before the operation, eleven years later. Its peculiarity consisted in the fact

that it was composed of several dozen lobes, separated from each other by deep furrows, which gave to the mass the same appearance as the surface of the brain. At a distance of from 0.5 to 1.0 cm., there were a few isolated lobules. The patient recovered rapidly from the operation and when seen more than a year later was in excellent health, having gained 8 Kg. in weight.

HEALING PROCESSES IN PULMONARY TUBERCULOSIS. P. HUEBSCHMANN, Beitr. z. Klin. d. Tuberk. **68**:718, 1928.

Small apical lesions of the acinous type heal frequently by the formation of a thin fibrous scar which fits itself into the surrounding alveolar structure. The neighboring alveoli, which have lost their elastic fibers, are usually emphysematous. This is the reason that such processes may not leave any roentgenologic signs. The disappearance of other roentgenologic shadows is frequently due to the absorption of perifocal exudate. Larger bronchopneumonic foci may heal only by encapsulation and fibrous replacement of the caseous center.

MAX PINNER.

THE ACTION OF CEMENT DUST ON THE LUNGS. F. SCHOTT, Beitr. z. Klin. d. Tuberk. **69**:43, 1928.

According to clinical and roentgenologic observations and experiments on rabbits, the following conclusions are reached: Inhalation of cement dust over a period of years may produce chronic catarrh, bronchitis and emphysema with alterations in the hilum. Actual disease does not occur before an exposure of at least ten years. Pneumonoconiosis produced by cement is more benign than chalicosis; it does not favor the development of pulmonary tuberculosis.

MAX PINNER.

THE DISTRIBUTION AND THE ORIGIN OF CONNECTIVE TISSUE IN PULMONARY TUBERCULOSIS. A. SEGA, Beitr. z. Klin. d. Tuberk. **69**:403, 1928.

Connective tissue is produced in the lung in three fundamentally different forms: (1) heavy and dense sclerosis with rich vascularization; (2) nodular fibrosis, and (3) annular fibrosis. The connective tissue arises from interalveolar septums and from the interlobular, subpleural, perivascular and peribronchial tissue. The genesis from interalveolar septums and from interlobular connective tissue occurs chiefly in annual and nodular fibrosis. The other types of origin lead chiefly to vascularized sclerosis.

MAX PINNER.

AN UNUSUAL THYROID TUMOR. W. ROTTER, Centralbl. f. allg. Path. u. path. Anat. **42**:289, 1928.

A woman, aged 41, had two masses the size of hen's eggs removed from the thyroid gland. Microscopically, these proved to be polymorphocellular sarcomas with spindle cells predominating. Scattered throughout were numerous adenomas and spots of necrosis. The lumens of the veins in the tumors were filled in many instances with sarcoma growths. Seven years later the woman broke her elbow and arm, and because of progressive swelling of the shoulder exarticulation was done. Gray-red tumor masses were found at the neck of the humerus and in the surrounding soft tissues. Microscopically, the tumor masses had the following components: colloid struma; adenoma trabeculare with solid cordlike strands; small and large epithelial collections, partly solid and partly cystic; papillary fibro-adenoma; cylindric cell carcinoma; squamous cell carcinoma with definite pearly formation, and spindle cell sarcoma. In accounting for the squamous cell elements, the author cites Klöpple, who saw such cell nests in the thyroid, and Wegelin, who saw squamous cell metaplasia in thyroid adenomas and the possibility of change in cell type in a metastasis.

GEORGE RUKSTINAT.

MELANOTIC PIGMENTATION OF THE EPICARDIUM. F. MATZDORFF, Centralbl. f. allg. Path. u. path. Anat. **42**:294, 1928.

The deposits were found in two patches varying in size from that of a linseed to that of a bean in the right ventricle in a man, aged 83; in multiple small dark-brown scale or shingle-like collections in the left ventricle in a woman, aged 59, and on the right ventricle of a man, aged 40. In the first and third instances, chromatophores were found in the endothelial layer of the epicardium and between it and the subepicardial fat tissue; in the second case, they were found between the epicardium and the muscle. According to the author, his is the first communication dealing with this pigment in the epicardium of man, but he points out the relative frequency of endocardial deposits in sheep in which a mixture of races has led to degenerative changes.

GEORGE RUKSTINAT.

EXTENSIVE MYOCARDIAL NECROSIS FOLLOWING ILLUMINATING GAS POISONING. H. TESSERAUX, Centralbl. f. allg. Path. u. path. Anat. **42**:344, 1928.

A woman, aged 69, found unconscious from illuminating gas poisoning, was given metrazol and lobelin, artificial respiration by the Draiger apparatus and relieved of 350 cc. of blood by venesection. Three days later she died, and at the autopsy there were found: confluent bronchopneumonia of the left lower lobe; symmetrical gray-brown spots of softening in the globus pallidus and suggillations and petechial hemorrhages mingled with light yellow spots of necrosis in the left ventricle of the heart. Microscopically, the myocardium exhibited loss of nuclei and eosinophilic staining; single muscle cells and bundles swollen and without cross-striations and in the yellow places medium sized fat droplets. Collections of polymorphonuclear leukocytes bordered such places. The blood vessel walls were everywhere normal, and bacteria were not seen in gram stains.

GEORGE RUKSTINAT.

THE STRENGTH OF THE CAPILLARY WALL AND CAPILLARY HEMORRHAGES. M. VOLTERRA, Klin. Wchnschr. **7**:1464, 1928.

The idea that the strength of the normal capillary is dependent on the endothelial cells does not rest on an anatomic basis. Fragility of the endothelial cells does not cause rupture of the capillary wall and capillary hemorrhage. An envelope of connective tissue, which is an essential part of the capillary wall, provides the resistance against rupture. The anatomic basis for capillary fragility or for any capillary hemorrhage is an injury of the connective tissue adventitia of the capillary.

AUTHOR'S SUMMARY.

CHANGES IN THE KIDNEYS IN DIABETIC COMA AND DEATH FROM UREMIA AFTER TREATMENT WITH INSULIN. E. J. KRAUS and H. SELYE, Klin. Wchnschr. **7**:1627, 1928.

Autopsy on three patients showed the kidneys to be swollen, yellow as with fatty changes and moist as though edematous. The histologic examination confirmed the general diagnosis of fatty changes in the kidneys of one patient, but in the other two patients, these were practically absent. There were hydropic changes and necrosis of the convoluted tubules. The anemia, especially that of the cortex, was especially noteworthy. The authors believe that after treatment with insulin the diabetic coma became a uremic coma, and that the kidney changes probably were an early stage of a glomerulonephritis.

E. F. HIRSCH.

CONGENITAL DEFECTS OF THE RADIUS AND EAR AND FACIAL PARALYSES. ERIK ESSEN-MÖLLER, Ztschr. f. Konstitutionslenhre **14**:52, 1928.

In a girl, aged 6 weeks, the author records the occurrence of asymmetry of the face due to paralysis on the right side associated with furrowed, blunt tipped tongue, apical attachment of the frenulum, bilateral ranula, absence of the muscles

of the left ear, absence of the left auditory canal, and deformity of the left upper extremity consisting of kinking of the humerus, ankylosis of the elbow and terminal joint of the third left finger, complete absence of the radius and the phalanges of the first two fingers. The variations in facial expression due to the paralysis are portrayed. The left upper extremity is carried bent at the elbow with a radial deflection and pronation of the hand. A review of 200 instances in the literature is included. Some of the small bones of the wrist are absent in most instances. Defects of the ear were present in 20; facial paralysis was observed in 100. The latter condition is regarded as a disturbance of the peripheral neuron. The frequency of the occurrence of this condition in the Women's Clinic was 4 among 17,000 new-born infants. The condition may be bilateral. The etiology is somewhat indefinite; internal and external causes such as heredity, phylogenetic radial developmental defects, solitary and multiple genetic irregularities, as well as intra-uterine factors of pressure, faulty embryo enclosures and toxic substances were discussed. The author is inclined to disregard the external factors as causative and holds that, in his case and in most of those in the literature, the etiologic agency is concerned with one or more developmental arrestments in the embryo.

FRANK R. MENNE.

THE SHRINKAGE OF PULMONARY TISSUE. F. KRAMPF, Ztschr. f. Tuberk. **51**:35, 1928.

Shrinkage of pulmonary tissue is observed following pneumonia of varying etiology, after inhalation of dust, in tuberculosis and syphilis and in pulmonary abscess. All these processes are considered under the name of "primary inflammatory cirrhosis." Secondary inflammatory cirrhosis follows the inflammation of the bronchial system in so-called congenital bronchiectasis, in bronchial stenosis and in bronchial occlusion. Pulmonary cirrhosis may be produced artificially by the following procedures: ligation of branches of the pulmonary artery, more pronounced by the simultaneous ligation of the pulmonary veins; embolic occlusion of a branch of the pulmonary artery and simultaneous ligation of pulmonary veins. The consequences of pulmonary cirrhosis are as follows: local emphysema, secondary bronchiectasis and compensatory emphysema in the unaffected parts. Any cirrhosis concomitant with pleural adhesions causes shifting of the mediastinal structures, elevation of the diaphragm, deformity of the thorax and the vertebral column and peripheral stasis. The right side of the heart becomes hypertrophied and dilated, which, in its turn produces the well known clinical symptoms. Experimental work led to the following practical conclusions: The ligation of branches of the pulmonary artery is successful only in a limited number of cases of bronchiectasis. The simultaneous ligation of arterial and venous branches is impracticable on account of the frequent and severe nutritional disturbances. Ligation of pulmonary veins may be promising in some cases.

MAX PINNER.

HEALING OF WOUNDS OF THE RENAL PELVIS AND URETER. Z. KAÏRIS, Ztschr. f. Urol. **21**:615, 1927.

Nephrotomy and pyelotomy are both used to remove stones from the renal pelvis. Each method has advocates who emphatically condemn any other. There has also been controversy over the way the wounds of the pelvis and ureter should be repaired after the stones are removed. Some operators use one type of suture, some another, and the operation in which a fibrous flap from the renal capsule or some of the perirenal fat is sewed over the wounds has proponents.

Kaïris reports uneventful, spontaneous healing of such wounds, some quite large, purposely left unrepaired in fourteen patients with stones in either the ureter or the kidney pelvis, and in dogs similar wounds of the ureters healed, also without suturing, so that even microscopically there was scarcely any trace left.

E. R. LE COUNT.

PERITONEAL PSEUDOMYXOMAS ORIGINATING FROM VERMIFORM APPENDIX. J. NAESLUND, Upsala Läkaref. Förh. **34**:1, 1928.

By tying the appendix off from the rest of the intestinal canal in new-born rabbits, Naeslund succeeded, within an observation period of 595 days, in producing well developed, large, distended formations filled with mucus (mucoceles), sometimes with diverticula. In a part of the cases, the mucoceles ruptured, the mucus in some instances reappearing later in pea-sized formations with epithelial cells, partly free, partly pedunculated, in the omentum. Small masses containing mucus and covered in places with epithelial cells were found also on the serous membrane of the small intestine. Pathologico-anatomic investigations were made in twelve cases of peritoneal-pseudomyxomas which originated from the appendix in man and which were all found by accident; nine occurred in men, three in women, making a total published to date of forty-eight cases in men and forty-one in women. His results agree in the main with those previously published. The changes which he produced experimentally in rabbits coincided essentially with those found in peritoneal pseudomyxomas in man. There are 136 illustrations and nine pages of bibliography.

CASE OF UTERINE ANGIOMYOMA. E. EHNMARK, Upsala Läkaref. Förh. **34**:219, 1928.

Two uterine tumors were found in Ehnmark's case, the larger one a fibromyoma with red necrosis, the smaller one an angiomyoma. A full description is given.

DIFFUSE XANTHOMATOSIS OF SMALL INTESTINE. H. KEY, Upsala Läkaref. Förh. **34**:247, 1928.

Key reports in detail a peculiar case with localization in the small intestine, discovered accidentally at necropsy in a woman, aged 67. He reviews the cases of xanthomatous changes in the stomach and intestinal canal published in the literature.

SUPRARENAL TUMORS. N. GELLERSTEDT and R. HJELM, Upsala Läkaref. Förh. **34**:271, 1928.

Roentgen examination in Gellerstedt and Hjelm's case, in a boy, aged 6, revealed a coral-shaped area of calcification above the left kidney. At necropsy, this was seen to belong to a tumor in the right suprarenal body, probably developed from the suprarenal cortex. The tumor consisted of a large number of sympathetic ganglion cells and nerve fibers, and was believed to be a ganglioneuroma. Because of the presence of many young and immature cells and the structure of the nervous tissue, it was classified as a ganglioblastoma.

MULTIPLE MYOMA OF COLON. K. G. KLING, Upsala Läkaref. Förh. **34**:307, 1928.

At the necropsy of a patient, aged 67 years, in whom a diagnosis of chronic colitis had been made, an extensive development of polypoid and sessile myomas, about the size of a hazelnut, was found in the lining of the entire large intestine. The tumors appeared to have developed from the muscularis mucosae.

CASE OF CIRCUMSCRIBED MYOSITIS OSSIFICANS IN BRACHIAL MUSCLE. S. ARNELL and R. RUBEN, Upsala Läkaref. Förh. **34**:333, 1928.

A round, hard tumor, without tenderness, proximal to the elbow fold, was noted in Arnell and Ruben's patient, one month after injury to the elbow. Roentgen examination revealed a calcified area, the size of a hen's egg, which was diagnosed as an ossified hematoma in the brachial muscle. Three months after the injury, the tumor began to decrease in size, and the bony structure became pronounced. Treatment consisted of massage and exercise during the first month after

the accident, after which the patient was able to work. In seven and a half months, the elbow function was completely restored and the osteoma was smaller. A month later, it was still further reduced in size and had the shape of a disk.

PATHOGENESIS OF HYDROCEPHALUS. ARVID LINDAU, Arch. path. et microbiol. Scandinav. **5**:25, 1928.

A summary is given of the formation, circulation and absorption of the cerebrospinal fluid (chiefly based on American investigations). On this basis, the nature and pathogenesis of hydrocephalus is discussed. Hydrocephalus is not a disease but a symptom of an occlusion in the circulating system of the cerebrospinal fluid. The author's method of injection of a dye at autopsy into the lateral ventricles to determine the site of the obstruction is described. This method may be employed as well in communicating as in noncommunicating hydrocephalus. Fifteen personally observed cases of hydrocephalus analyzed according to Dandy's principles are presented; among them is a case of external hydrocephalus.

AUTHOR'S SUMMARY.

HYPERPLASIA OF THE THYMUS WITH ENLARGEMENT OF THE PARATYROIDS. H. BERGSTRAND, Acta path. et microbiol. Scandinav. **5**:552, 1928.

In a woman, aged 48, who died from cerebral hemorrhage, the thymus weighed 30.5 Gm. and the parathyroids together weighed 1,370 mg. The enlargement of the parathyroids was due to newly formed parenchyma. The significance and relationship of these enlargements remain obscure.

INTRA-ABDOMINAL HEMORRHAGE FROM RUPTURE OF CORPUS LUTEUM OF OVARIAN PREGNANCY. FRANCIS HARBITZ, Norsk. Mag. f. Laegevidensk. **89**:866, 1928.

A case is described of hemorrhage into the abdominal cavity from rupture of a corpus luteum in which typical chorionic villi were found. The case illustrates that rupture of corpus luteum often is due to ovarian pregnancy, but in a previous study of fifteen or twenty instances, Harbitz did not detect any products of pregnancy.

Pathologic Chemistry and Physics

THE VOLUME OF THE BLOOD. D. C. DARROW and T. E. BUCKMAN, Am. J. Dis. Child. **36**:247, 1928.

In infants with dehydration and in others with water retention, there is a tendency to reduction of the plasma volume. As the clinical picture improves in both types of cases, there is a return to the normal in the absolute plasma volume and the volume of plasma in cubic centimeters per kilogram of body weight. In subacute and chronic cases, the condition is one of oligemia rather than of anhydremia. In cases of dehydration, the loss of serum protein is generally proportionally less than the loss of plasma; in cases of retention of water, the loss of serum protein is generally proportionally greater than the loss of plasma volume. Thus the concentration of the serum protein is not a trustworthy index of dehydration or water retention, or of changes in blood volume. The concentration of the crystalloids is only slightly affected by the change in plasma volume.

H. E. LANDT.

THE INFLUENCE OF FEEDING ON CERTAIN ACIDS IN THE FECES OF INFANTS. J. R. GERSTLEY, CHI CHE WANG, R. E. BOYDEN and A. A. WOOD, Am. J. Dis. Child. **36**:289, 1928.

The addition of an excess of lactose to breast milk causes little change in the excretion of the volatile acids, the titratable acid or the weight of the stools. The

addition of an excess of lactose to mixtures of modified cow's milk causes only slight change in the total excretion of the volatile acids, though it does decidedly increase the amount of free acid. The frequency and weight of the stools are also affected. The addition of 12 per cent lactose created a relationship of total titratable to total volatile acidity resembling that in breast milk. This may be due to the similar relations existing between lactose and protein in this formula. An excess of lactose in either breast milk or cow's milk did not result in an immediate disturbance of nutrition. An intercurrent bronchitis was a much more powerful factor in increasing the excretion of acid than any increase of carbohydrate in the mixtures. Following such a parenteral infection, the excretion of acid remained high for several weeks, although the infant remained clinically well. The addition of an excess of lactose at this time did not cause an immediate disturbance. These studies favor the Finkelstein hypothesis that intestinal fermentations are not primary but occur only after the development of some disturbance of nutrition.

AUTHORS' SUMMARY.

COMPOSITION OF BONE. M. J. SHEAR and B. KRAMER, J. Biol. Chem. **79**:105, 121, 125 and 147, 1928.

A technic is described for the analysis of minute amounts of pathologically calcified tissue. The methods presented have permitted the examination of such specimens as calcified, tuberculous lymph nodes, the calcified cusp of an aortic valve, etc. The Ca:P ratio was not found abnormal except in specimens of calcified fibroid of the uterus. The physicochemical mechanism of the calcification process is discussed.

ARTHUR LOCKE.

ON THE TIME OF ABSORPTION AND EXCRETION OF BORIC ACID IN MAN. L. KAHLENBERG and N. BARWASSER, J. Biol. Chem. **79**:405, 1928.

Boric acid passes through living skin with remarkable ease and rapidity. The carefully cleaned feet of three adult men were immersed in a saturated, aqueous solution of boric acid, held at 45 C. Samples of the urine were drawn from each subject at intervals of five and fifteen seconds and examined for the presence of borates. Traces were found, invariably, within from fifty to fifty-five seconds of the moment of immersion.

ARTHUR LOCKE.

VITAMIN A DEFICIENCY AND CALCIFICATION OF THE EPITHELIUM OF THE KIDNEY. E. C. VAN LEERSUM, J. Biol. Chem. **79**:461, 1928.

The calculi found in the bladders of rats maintained on a diet deficient in vitamin A probably develop from nuclei washed down from the tubules of the kidneys. The nuclei may be observed microscopically as crystalline concretions. The shape of these concretions, their possession of a kind of nucleus, their position against the membrana propria and their arrangement in a circle suggest that they may represent epithelial cells undergoing calcification.

ARTHUR LOCKE.

ARTEFACTS AS A GUIDE TO THE CHEMISTRY OF THE CELL. C. E. WALKER, Proc. Roy. Soc. London **103** B:397, 1928.

The methods of fixation and staining which are used for the demonstration of "Golgi bodies" have been applied to emulsions of methyl myristate and laurate in mediums containing unsaturated lipids such as lecithin and cephalin. The descriptions of the final preparations appear to support the hypothesis that the Golgi elements are separations of cell lipid, produced during the drastic fixation processes necessary for their demonstration.

ARTHUR LOCKE.

Microbiology and Parasitology

CLOSTRIDIUM WELCHII SEPTICEMIA COMPLICATING PROLONGED LABOR DUE TO OBSTRUCTING MYOMA OF UTERUS. P. TOOMBS and I. MICHELSON, Am. J. Obst. & Gynec. **15**:397, 1928.

In addition to a detailed report of their own case, the authors collected from the literature the reports of forty-one cases of puerperal sepsis due to *Clostridium welchii*. There are two main groups, the abortion group comprising 60.97 per cent, and the nonabortion group, 39.03 per cent, of the total cases. The latter group was divided into subgroups which can include any possible condition that delays the progress of the child through the birth canal. The introduction of the organism is favored by too frequent vaginal examinations. Criminal abortions or operative procedures favor the implantation of the organism in the first group.

A. J. KOBAK.

SEX DIFFERENCES IN THE PATHOLOGIC PICTURE OF SYPHILIS. A. SCOTT WARTHIN, Am. J. Obst. & Gynec. **15**:595, 1928.

Latent syphilis is a common form of the disease in the female. If the spirochete enters through the cervical or uterine columnar cell mucosa, the primary lesion may be absent or may escape detection entirely, being smaller and less indurated than in the male, and the diagnosis may be missed. When present on stratified epithelium of the cervix, clitoris, urinary meatus or nipple, it is frequently mistaken for carcinoma. The skin lesions of the secondary stage are milder, or may be entirely absent, but there is a greater preponderance of constitutional symptoms, particularly arthritic manifestations, during this stage. In the majority of women, late syphilis is latent during the child-bearing period, accompanied by apparent good health and a negative Wassermann reaction; nevertheless, these patients may bring forth syphilitic children with involvement of all the fetal adnexa. The central nervous system, heart and aorta less frequently are involved, and the ovary is practically immune to the lesion. On the contrary, the liver, pancreas, suprarenals and rectum are more prone to spirochetal invasion. There is, therefore, a relatively greater immunity in the female, and the general aspects of the disease appear milder. At the menopause, signs of syphilis and a positive Wassermann reaction may be present. While there is a general modification of the disease as a whole, in the female, there is, in addition, a marked resistance of certain organs to the spirochete.

A. J. KOBAK.

SUBACUTE BACTERIAL ENDOCARDITIS OF STREPTOCOCCUS VIRIDANS TYPE IN PREGNANCY, WITH TWO CASE REPORTS. H. G. WALSER, Am. J. Gynec. & Obst. **15**:840, 1928.

Two cases of typical subacute bacterial endocarditis are reported; the blood cultures taken during pregnancy revealed *Streptococcus viridans*. The clinical manifestations were of the usual type. In one case, after a full term delivery, the blood from the umbilical cord was positive for *Streptococcus viridans*, although the child did not show subsequent clinical disturbance, and the placenta was apparently normal. In the other case, blood cultures obtained from the mother during pregnancy showed *Streptococcus viridans*, and throat culture a predominating nonhemolytic streptococcus. The infant died seventeen hours after premature birth, with definite signs of intra-uterine infection, transmitted apparently through the placenta. Postmortem examination of cultures from the heart blood showed a nonhemolytic streptococcus; in addition, there were multiple infarctions of the brain. Postmortem examination of the mother was not made. Thus Walser showed that the fetus can be infected in maternal infection. The course of the disease or pregnancy was not particularly affected

A. J. KOBAK.

THE PATHOLOGY OF YELLOW FEVER IN MAN AND MONKEY. N. PAUL HUDSON, Am. J. Path. **4**:395, 407 and 419, 1928.

The lesions in *Macacus rhesus*, brought about by experimental infection with yellow fever virus, seem to result from a severe intoxication, as in the case of yellow fever in man, recently expressed by Kiotz and Simpson. Neither in the monkey nor in man is there any evidence of the localization of the virus. We have been unable to find any constant bacterial form, or leptospiras or spirochetes demonstrable in Levaditi preparations either in cases in human beings or in experimental tissues. The specimens of the liver from the monkey tend to confirm the fact that necrosis in the liver is essentially midzonal in type in yellow fever, with less altered cells increasing toward the periphery of the region involved. Likewise, as in cases in man, when the degenerative changes approach the limiting structures of the lobule, the most extreme necrosis is usually in the midzone. Klotz and Simpson have recorded that in the spleen there is a sequence of changes involving the lymph follicles, from early enlargement of the follicle due to hyperplasia of the endothelial elements, followed by loss of lymphocytes, to final degeneration of the endothelial cells. We have not observed the first stage given in tissues from man or from monkey; otherwise, a study of the rhesus sections makes it evident that the process described by these workers is probably correct. A stage of necrosis is obvious in the monkeys, but is uncommonly seen in the spleens of human beings, in which it is possible the stage might have been passed at the time of death. We would add, however, that in monkeys, degeneration and necrosis involves the lymphoid as well as the endothelial elements of the follicles. The fatty degeneration of the fibers of the heart muscle and the same and other acute degenerative changes of the kidney in the rhesus monkey add to the evidence for the clinical manifestations of this disease in man. If experience bears out the hope that *M. rhesus* is regularly susceptible to the yellow fever virus, this animal will prove to be of incalculable value in the diagnosis of yellow fever in man because of the remarkably accurate reproduction of gross and microscopic lesions.

AUTHOR'S SUMMARY.

MYOCARDIAL DEGENERATIONS IN YELLOW FEVER. D. E. CANNELL, Am. J. Path. **4**:431, 1928.

Cloudy swelling and granular and fatty degeneration were found constantly in the hearts of both the cases in man and those experimentally induced in *Macacus rhesus*. Primary inflammatory changes were not seen in the heart in yellow fever. Secondary response of the white blood cells to intense degeneration was observed in two cases in human beings. The distribution and intensity of granular and fatty degeneration were patchy and variable both in cases in man and those in *Macacus rhesus*. Fatty degeneration was most marked in the neighborhood of the nuclei of the fibers. The causation of the slow pulse in yellow fever is still uncertain, and doubt is thrown on the belief that it is due to the jaundice. Further investigation of the clinical function and the pathologic changes in the bundle of His may lead to the solution of the problem. The lesions in the hearts of human beings and in those of *Macacus rhesus* are essentially the same. The lesions in the heart are in themselves not sufficient to make a diagnosis of yellow fever.

AUTHOR'S SUMMARY.

DISSOCIATION OF THE RAWLINGS STRAINS OF B. TYPHOSUS. NEWTON W. LARKUM, Am. J. Pub. Health **18**:647, 1928.

As a result of reports that growth of R types in homologous immune serum resulted in reversion to S types, studies of the nature of the Rawlings strains of *B. typhosus* were carried out in order to determine the state of the culture and the extent to which dissociation might be controlled. It has been generally accepted that the R type was avirulent or less virulent than the corresponding S type. Hence, if the strains of *B. typhosus* used in vaccines to produce immunity should

be of the R type, the antiserum resulting would tend to convert the less virulent organisms entering the individual, if infection occurred with such types, into more virulent forms and, furthermore, would tend to prevent the usual dissociation in case of infection with a virulent organism. The desirability of using only S type cultures to produce immunizing vaccines is thus emphasized.

A study of six strains of *B. typhosus,* Rawlings, actually in use in as many laboratories in the United States, reveals that in only one case is an S strain in use. It is recommended that — since animal passage can usually change an R to an S type — laboratories making vaccines follow this procedure.

BACTERIUM ABORTUS INFECTION IN MAN. JAMES G. MCALPINE and FRIEND LEE MICKLE, Am. J. Pub. Health **18**:609, 1928.

Ten thousand, one hundred and fifty-seven samples of serums from human beings, which were submitted for the Wassermann examination, were tested by the agglutination reaction, *Bacterium abortus* being used as the antigen. Of this number, approximately 0.6 per cent gave reactions up through the 1-100 dilution. These results, which were obtained in a state in which preliminary testing has shown at least 90 per cent of the dairy herds to be infected with *B. abortus* and approximately only 60 per cent of the milk to be pasteurized, indicate that the infection of man with the bovine type of *B. abortus* is relatively rare, provided any significance can be attached to the agglutination test. Attempts to follow the small number of cases reacting through the 1-100 dilution by the agglutination test were made through questionnaires, subsequent testing and blood cultures. This method was unsatisfactory on the whole, as many of the patients remained under the care of the attending physicians for only a short time.

AUTHORS' SUMMARY.

TORULA INFECTION IN MAN. O. BERGHAUSEN, Ann. Int. Med. **1**:235, 1927.

The literature affords twenty-four examples of torula infection in man, to which Berghausen adds a case. *Torula histolytica* is a member of the group of blastomycetes known as *Fungi imperfecta; Oidium, Monilia* and *Dematium* comprise the other members of the group. In Berghausen's case, *Torula histolytica* was isolated from a large ulcer of the tongue. The patient died. There was some clinical evidence of involvement of the lungs, but postmortem confirmation could not be made, since autopsy was refused.

WALTER M. SIMPSON.

CHRONIC ULCERATIVE COLITIS. H. W. SOPER, Ann. Int. Med. **1**:313, 1927.

Soper isolated the Bargen-Logan diplococcus in all of sixteen cases of chronic ulcerative colitis in which a search for the organism was made. The conclusion is reached that the Bargen-Logan diplococcus is the primary etiologic agent in this disease.

WALTER M. SIMPSON.

THE HISTOPATHOLOGY OF THE TONSIL IN ACUTE RHEUMATIC FEVER AND CHOREA. W. W. G. MACLACHLAN and DEW. G. RICHEY, Ann. Int. Med. **1**:506, 1928.

Maclachlan and Richey attach importance to the perivascular lymphoid and plasma cell lesions found in the capsule and pericapsular tissues of the faucial tonsil in fourteen of eighteen cases of rheumatic fever and in four of five cases of chorea. Endothelial proliferation of the lining cells of the capillaries and perivascular lymph spaces was observed in eleven instances, and in six cases the endothelial cells were multinucleate and associated with granulomatoid lesions, suggesting the so-called Aschoff nodules. The writers hesitate to state that such lesions in the tonsil are specific for rheumatic fever or chorea.

WALTER M. SIMPSON.

SOME CONSIDERATIONS UPON THE ETIOLOGIC AGENT IN YELLOW FEVER. ARISTIDES AGRAMONTE, Ann. Int. Med. **1**:977, 1928.

Agramonte concludes that the etiologic agent of yellow fever has not been demonstrated. The claims of Noguchi and other investigators that *Leptospira icteroides* is the specific germ of yellow fever have been conclusively disproved because: (*a*) *L. icteroides* and *L. icterohemorrhagiae* show crossed serologic reactions indicating their identity. (*b*) Serum from patients convalescing from yellow fever does not protect against *L. icteroides,* while serum from patients convalescing from Weil's disease does protect against both *L. icteroides* and *L. icterohemorrhagiae.* (*c*) *L. icteroides* gradually increases in numbers in the blood of inoculated animals, while the real yellow fever germ disappears from the circulating blood at the third or fourth day. (*d*) *L. icteroides* fails to infect mosquitoes so that in due time they may infect man. (*e*) *L. icteroides* can penetrate the unbroken skin and produce infection, while yellow fever has been shown to be noncontagious, even through cuts or abrasions of the skin. Any vaccine or serum prepared with the *L. icteroides* cannot be of value, either protective or curative, as regards yellow fever.

WALTER M. SIMPSON.

METADYSENTERY. A. CASTELLANI, Ann. Int. Med. **2**:155, 1928.

Organisms of the metadysentery group have been found in many clinical conditions, with either simple diarrhea or dysenteric diarrhea. The paper directs particular attention to a chronic type of colitis characterized by recurrent attacks of diarrhea, generally simple, but occasionally dysenteric. The metadysentery bacilli are intestinal organisms similar to the dysentery bacilli, Shiga and Flexner, in that they do not produce gas in any sugar. They differ, however, in that they clot milk or produce acidity in lactose; they clot milk without producing distinct acidity in lactose, or they produce acidity in lactose without clotting milk. Some species (*Lankoides-Dysenteroides* group) are pathogenic, while others probably are not. *Bacillus metadysentericus* is pathogenic. In the chronic conditions, the blood generally contains a large number of agglutinins for the variety of metadysentery bacillus isolated from the patient's stools.

WALTER M. SIMPSON.

SECOND INFECTION IN SYPHILIS. C. R. L. HALLEY and H. WASSERMAN, Arch. Int. Med. **41**:843, 1928.

A statistical study has been made of case reports in the literature since 1910 of second infection with syphilis, and to this number there are added eight new cases from the Johns Hopkins Hospital Clinic. The total number of cases accepted is 237, while 447 were rejected as not fulfilling the condition adapted for this study. Two hundred and thirty-two of these patients, or 97.8 per cent, were treated for the first infection during the primary or secondary stage. The duration of infection before the first treatment is known in eighty-three cases. Practically all of these were first treated before the end of sixteen weeks, and sixty-eight before the end of eight weeks. Second infection occurred in four patients, or 1.6 per cent, who had syphilis in a latent or late form at the time they were first treated for the disease. Second infection occurred in one patient, or 0.4 per cent, who presented undoubted evidence of congenital syphilis. The expressed relation of the time of treatment of the first infection in man and the incidence of second infection is paralleled closely by data collected on 328 rabbits experimentally injected with syphilis. It is indicated that the duration of a first infection before treatment has an important bearing on the patient's acquired resistance to a second infection.

H. R. FISHBACK.

MICROBIC DISSOCIATION IN STREPTOCOCCI. L. O. DUTTON, J. Bact. **16**:1, 1928.

As a basis for future work some of the more common variations of the colonies in streptococcal colonies have been described. Little is clear concerning the sequence

of the types of colonies, but it is apparent that most of the variations of the colonies that have been described for other bacterial species occur also in this group. The S type colony is clearcut, while the R type is not so definite. There are many intermediate types. In fluid mediums agglutination is associated with the R types. Certain variations also parallel the types of colonies, diplococcic and gram-positive, regular cells being of the S type; while long-chained, irregular, gram-variable forms are of the R type. In natural infections, virulence seems also to parallel the types, the S type being virulent, and the R types being relatively avirulent.

AUTHOR'S SUMMARY.

PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF LEPTOSPIRA. I. J. KLIGLER and M. ASHNER, J. Bact. **16**:79, 1928.

Morphologically, *Leptospiras* are exceedingly variable, the form and size depending on the medium, temperature and rate of growth. These organisms carry a positive charge at ordinary reactions, but at p_H 9.2 they become negatively charged. The important distinguishing characters which differentiate *Leptospira* from some of the true viruses are sedimentability and filtrability. *Leptospira* may be sedimented by prolonged centrifugation at 3,000 revolutions. From four to five hours seem to suffice practically to free a suspension of these organisms. Although they pass readily through a Berkefeld or Seitz filter, only a relatively small proportion of the organisms pass through. *Leptospiras* are readily cultivable on artificial mediums. The most satisfactory procedure for primary cultivation from infected animals is to cover the blood-free fibrin clot, or the sedimented red cells, with a small amount of Noguchi's leptospira medium (to a height of from 1.5 to to 2 cm.). *Leptospiras* are obligatory aerobes and the pathogenic forms die promptly when placed under anaerobic conditions.

AUTHORS' SUMMARY.

THE ACTION OF IRON AND CITRATE IN SYNTHETIC MEDIA FOR TUBERCLE BACILLI. GUILFORD B. REED and CHRISTINE E. RICE, J. Bact. **16**:97, 1928.

Data have been presented to show that the addition of iron in the form of chloride or sulphate to a synthetic medium, consisting of asparagin, glycerol, disodium acid phosphate and sodium chloride over the ordinary p_H growth range of tubercle bacilli, results in almost, though not quite, complete precipitation of the iron. The addition of sodium citrate, probably through the formation of complex ions, inhibits the precipitation of iron over this p_H range. In a synthetic medium adjusted to p_H 7.4, it has been shown that tubercle bacilli produce approximately 20 per cent more growth when iron and no citrate is added, and slightly over 100 per cent more growth when both iron and citrate are added to the medium. In more acid and more alkaline mediums less conspicuous advantages result from the addition of iron. Similar results are obtained with the growth of *B. phlei* and *B. leprae* in these mediums.

AUTHORS' SUMMARY.

VARIATIONS OF STREPTOCOCCI WITH A NOTE ON HEMOLYSIN PRODUCTION. M. FROBISHER, JR., and E. R. DENNY, J. Bact. **16**:109, 1928.

For the study and proper classification of streptococci in blood agar the following are essential: The differentiation of types based on low power microscopic observation of deep and not of surface colonies in blood agar plates. The use of tube hemolysin tests with properly prepared cultures to supplement plate observations. Genuine, alpha type streptococci have frequently been encountered which possess marked hemolytic powers, producing hemolytic zones from 2 to 4 mm. wide and appearing, to the naked eye, to be beta type (hemolyticus) streptococci. These strains never caused hemolysis when their broth cultures were mixed with red cell suspensions. When the deep colonies of such streptococci in blood agar pour plates were viewed with the low power microscope, they were seen to have about them, inside the hemolytic zone, the zone of methemogobinized cells common to all alpha type streptococci. A number of such streptococci have been found to

lose a large part (not all) of their hemolytic powers after being kept for some time under various artificial conditions, and to appear like the ordinary and easily recognized alpha type of streptococcus. The diminution of hemolytic properties may be due to a change in the organisms or to some obscure variation in technic. It is suggested that some of the reported mutations in which a beta type streptococcus has been said to change into an alpha type streptococcus may be due to failure to recognize the true character of the organism in the first place, accompanied by a diminution of the original hemolytic properties. It is possible that many of the anomalous results of many sorts reported by investigators of streptococci may be due to confusion in the identification of the alpha and beta types.

AUTHORS' SUMMARY.

STREPTOCOCCUS VIRIDANS DERIVED FROM SINGLE CELL STRAINS OF STREPTOCOCCUS HEMOLYTICUS. FRANCIS B. GRINNELL, J. Bact. **16**:117, 1928.

Single cell strains of hemolytic streptococci may under certain conditions of cultivation give rise to daughter strains of the alpha type. These alpha variants resemble *Streptococcus viridans* in every respect. The change from the beta to the alpha type occurs only with certain strains. The change is characterized by the occasional appearance of alpha type colonies, and there is not as a rule a complete change of the whole culture to the alpha type similar to the change from S to R with pneumococci. The alpha variants remain true to type with no tendency to revert under the usual conditions of cultivation. The loss of hemolytic power is accompanied by a slight loss of virulence. The alpha derivatives usually but not always give the same fermentation reactions as the beta strains from which they are derived.

AUTHOR'S SUMMARY.

HEMOLYSIS BY PATHOGENIC BOVINE STRAIN OF B. COLI. MARION L. ORCUTT, J. Bact. **16**:123 and 135, 1928.

Studies on the hemolytic properties of one strain of *B. coli* isolated from a bovine source showed that the hemolytic power parallels the number of living organisms present in culture, but that this is not the case in the presence of inhibiting factors. Veal infusion broth inhibited hemolysis without inhibiting growth, but this power became less marked when the broth was filtered or diluted. In certain concentrations peptone alone had an inhibitory effect on hemolysis.

In the second report, inhibition of hemolysis was demonstrated in normal serums from the horse and cow, and from man. Cholesterol alone produced some inhibition of hemolysis but the concentration of this agent did not parallel the degree of inhibition. Specific immune serum prevents hemolysis more than normal serum. The author concludes that the inhibiting power of any given serum on hemolysis may be due to a combination of factors including cholesterol, protein composition and specific antibody content, but that the different inhibiting values of different normal serums seem to be related chiefly to their protein composition.

EDWIN P. JORDAN.

A COMPARATIVE STUDY OF THE ACTION OF SODIUM RICINOLEATE ON BACTERIA. ANTHONY KOZLOWSKI, J. Bact. **16**:203, 1928.

Among the streptococci investigated, those isolated from cases of erysipelas, measles and scarlet fever were the most susceptible to the bactericidal action of sodium ricinoleate. They were killed by this soap in a dilution of 1:5,000 in about seven hours or sooner at 35 C.; in a control culture, without soap, they survived for more than one week; their growth was inhibited by this soap in a dilution of 1:20,000 or less; in their cellular susceptibility they approach that of pneumococci which were killed by the same soap, under similar conditions, in a dilution of 1:10,000. Pneumococci are dissolved quite readily by sodium ricinoleate in a dilution of 1:5,000; in this regard they differ from other bacteria investigated. Of the bacilli, *B. diphtheriae* was found to be quite susceptible to

the inhibitory, and much less to the bactericidal, action of sodium ricinoleate; *B. tuberculosis* showed a slight susceptibility, as it was inhibited in its growth by this soap in a dilution of 1:2,000; *B. paratyphosus, B. dysenteriae, B. coli communis* and *S. fecalis* are fairly resistant to the inhibitory and bactericidal action of sodium ricinoleate. They are probably adapted to the action of soaps in their natural conditions of growth. An intermediate place between susceptible and nonsusceptible bacteria is taken by some streptococci which are more or less saprophytic, i. e., *S. lacticus, S. pyogenes* and *S. viridans.* These were inhibited in their growth by sodium ricinoleate in a dilution of 1:5,000 or lower, but were not killed in a dilution of 1:1,000. The bactericidal effect of sodium ricinoleate is conditioned by the specific cellular susceptibility of the bacteria, and further by the concentration of the soap solution, by the time and the temperature of exposure. The difference in the cellular susceptibility of various strains of the groups of streptococci, isolated from cases of measles, scarlet fever and other infections, might be helpful in bacteriologic investigations. Sodium ricinoleate may be used instead of bile for the dissolution of pneumococci in diagnostic routine work.

AUTHOR'S SUMMARY.

BACTERICIDAL PROPERTIES OF THE ACYL AND ALKYL DERIVATIVES OF RESORCINOL. BETTYLEE HAMPIL, J. Infect. Dis. **43**:25, 1928.

From a detailed study of the alkyl and acyl derivatives of resorcinol and some of the intermediate ketones, the following are some of the chief results: An elevation of temperature from 20 to 37 C. increases the bactericidal action of all derivatives tested; in the presence of organic material at 37 C. both series tend to lose their bactericidal activity; in alkaline solution the alkyl resorcinols are increased in bactericidal activity beyond the hexyl compound, and the disinfecting power of these derivatives in general depends mostly on their solubility in water.

AUTHOR'S SUMMARY.

INFLUENCE OF CARBOHYDRATES ON BACTERIAL DECOMPOSITION OF UREA. MITSUTERU ISHIKAWA, J. Infect. Dis. **43**:67, 1928.

B. ammoniagenes, B. aerogenes, B. morgani, B. proteus vulgaris and *Staphylococcus aureus* actively decompose urea in culture mediums; the washed cells of these bacteria also possess a definite urea-splitting activity.

The presence of an utilizable carbohydrate accelerates the disintegration of urea by these bacteria, cultured and washed. Sodium formate added to the culture medium does not exert any increasing effect.

Thymol has an inhibitory influence on the liberation of ammonia from urea, by cultured or washed organisms, in the presence or absence of dextrose.

The symbiosis of an urea-splitting organism with a nonurea-decomposing bacterium, in a medium containing a carbohydrate fermentable by the latter organism but not by the former, increases the generation of ammonia, in comparison with the single culture of the urea-splitting organism.

AUTHOR'S SUMMARY.

BEHAVIOR OF BRUCELLA ORGANISMS TOWARD GENTIAN VIOLET. I. FOREST HUDDLESON and ELIZABETH ATELL, J. Infect. Dis. **43**:81, 1928.

Varieties, and strains within a variety, of the genus *Brucella* exhibit a difference in sensitiveness to gentian violet in a medium, expressed in degree of growth. The growth of strains of the variety of *B. melitensis* and *paramelitensis* is not inhibited on a medium by the presence of gentian violet in dilutions of 1:100,000 and 1:250,000. A few of the strains are slightly inhibited by the presence of a 1:50,000 dilution of the dye. Strains of the variety of *B. abortus* may be divided into two groups as regards their ability to grow on a medium in which gentian violet is present, namely, those which are markedly inhibited and those which fail to grow in the presence of 1:50,000 and 1:100,000 dilution of the dye. The

latter group contains one bovine strain, several human strains and all porcine strains in the possession of the writers. The difference in dye sensitivity may prove to be a means of distinguishing strains of the abortus variety not otherwise distinguishable.

AUTHORS' SUMMARY.

THE ACTION OF PHENOL AND FORMOL ON AEROBIC AND ANAEROBIC ORGANISMS. JOSEPH P. SCOTT, J. Infect. Dis. **43**:90, 1928.

It is shown that phenol in strengths up to 5 per cent acts slowly on anaerobic organisms, while formaldehyde in dilutions of from 0.5 to 0.75 sterilizes anaerobic cultures rapidly,

The greater susceptibility of aerobic spore-forming organisms to these disinfectants make it possible to eliminate aerobic contaminations from anaerobic cultures by the use of 0.5 per cent phenol or formol.

AUTHOR'S SUMMARY.

RICKETTSIA OF ROCKY MOUNTAIN SPOTTED FEVER IN THE BRAIN OF THE INFECTED GUINEA-PIG. HOBART A. REIMANN, J. Infect. Dis. **43**:93, 1928.

In the examination of guinea-pigs infected with Rocky Mountain spotted fever, *Rickettsia* were found in only one of eight brains, which was within a vein of the cerebrum and not in the brain substance. If the blood of the brain is responsible for the infectiousness of this tissue the question arises concerning the arbitrary infectious dose of the brain of animals killed by exsanguination. Because *Rickettsia* are small and are easily decolorized in the course of staining it is possible that they have been overlooked.

AUTHOR'S SUMMARY.

SURFACE TENSION IN RELATION TO BACTERIAL GROWTH: WITH SPECIAL REFERENCE TO LACTOBACILLUS. ALEXANDER ALFRED DAY and WILLIAM MERRIOTT GIBBS, J. Infect. Dis. **43**:97, 1928.

The effect of lowered surface tension on the development of *L. acidophilus* and *L. bulgaricus* was studied. The surface tension was depressed by applications of four soaps, together with sodium ricinoleate, sodium oleate and sodium taurocholate.

No instance was found in which inhibition of growth could be attributed to lowered surface tension.

Sodium ricinoleate exerted a toxic action toward both *L. acidophilus* and *L. bulgaricus*, but this action could not be used as a means of identifying the two organisms.

The fermentation of maltose, sucrose and levulose does not offer a means of differentiating *L. acidophilus* from *L. bulgaricus*.

AUTHORS' SUMMARY.

MONILIA FROM OSTEOMYELITIS. CHARLES L. CONNOR, J. Infect. Dis. **43**: 108, 1928.

A budding yeast-like organism forming reluctantly a mycelium in certain culture mediums and otherwise behaving as *Monilia* was isolated from an abscess of the buttock which communicated with the iliac bone. There had been a long-standing osteomyelitis of the humerus, and the organisms were seen in tissues from this lesion also. This strain of *Monilia* corresponds with no described species so far as fermentation tests are concerned, but it is pointed out that such tests are unreliable as a means of differentiating the group of organisms variously called *Torula, Cryptococcus, Blastomyces, Oidia, Monilia* or *Oospora*. A review of the characters by which these organisms are classified shows that they do not differ sufficiently to be given different names; that, in fact, they represent only two different types, namely, a mycelia-forming group (*Blastomyces, Oidia, Monilia, Oospora*, all classified as *Parasaccharomyces* by Anderson), and a

nonmycelia-forming group (*Torula* or *Cryptococcus*). These two groups with a third, *Mycoderma*, provide for all those yeast-like organisms known as *Fungi imperfecti*. The nature of these organisms does not permit at present of a more elaborate classification.

AUTHOR'S SUMMARY.

BACTERIAL ADAPTATION TO ACRIFLAVINE. VICTOR BURKE, CATHERINE ULRICH and DON HENDRIE, J. Infect. Dis. **43**:126, 1928.

Staphylococcus albus shows increased tolerance for the antiseptic dyes after from six to eight hours' exposure. This adaptation to the dyes is temporary and disappears when the organism is grown on dye-free agar. Delayed growth of the organism in the presence of the dye is due in part at least to the process of adaptation of the organism to the dye. The increased tolerance of the organism for the dye is partially specific. *Staphylococcus albus* can be separated into two strains which differ in their ability to tolerate neutral acriflavine.

AUTHORS' SUMMARY.

CHOICE OF ANTISEPTIC DYE IN MIXED INFECTIONS. VICTOR BURKE, M. P. JESSUP and SMITH PHILIPS, J. Infect. Dis. **43**:131, 1928.

Gentian violet is more bactericidal than neutral acriflavine for both the gram-positive and the gram-negative organisms commonly found in wounds. Blood reduces the bactericidal action of both dyes. No advantage is gained by mixing the two dyes. The dye most effective against the most resistant organism in the wound should be used.

Since bacteria acquire specific dye tolerance in a few hours rotation of dyes in the treatment of infections may be beneficial.

The bactericidal strength of neutral acriflavine varies with the manufacturer. Different samples from the same manufacturer are nearly uniform in bactericidal action.

AUTHORS' SUMMARY.

TRACHOMA STUDIES. IDA A. BENGTSON, Pub. Health Rep. **43**:2210, 1928.

The microscopic evidence in a study of film preparations and sections of conjunctivae and tarsi from cases of trachoma is that the inclusion bodies in the epithelial cells originate from rod-shaped micro-organisms which tend to occur as diplobacilli, and that the development of the inclusion body after the entrance of the rod into the cytoplasm of the cell is due to the multiplication of the organism and subsequent reaction on the part of the cell against the invading bacteria, transforming them into the small reddish-staining coccoid forms designated by von Prowazek as "elementary bodies" with the blue-staining "mantle" which represents the partially dissolved portion of the bacteria. The "free Lindner initial bodies" appear to be pleomorphic or modified forms of rod-shaped bacteria which have been seen rather infrequently outside the cells and which occur as blue-stained oval or cylindrical bodies stained at the rim and bipolarly. These are probably also formed as the result of the action of conjunctival fluids on the rod-shaped micro-organisms. In interpreting the inclusion body, the "elementary bodies" and the "free initial bodies," the fact must be borne in mind that they are found in the conjunctiva which, on account of its exposure, is particularly liable to the invasion of micro-organisms and must, therefore, be unusually rich in protective substances which are probably lytic in action. These act on the bacteria to destroy or inhibit their growth, and it is not surprising that the latter should be changed into forms which are quite unrecognizable when compared with those occurring in artificial culture mediums containing none of these substances antagonistic to their growth.

It has been possible to produce experimentally forms corresponding in appearance to the so-called "elementary bodies" of von Prowazek and the "initial bodies"

of Lindner, by the inoculation of certain gram-negative rod-shaped organisms isolated from the conjunctiva of trachoma cases, into the conjunctiva of guinea-pigs. A study of preparations made a short time after the inoculations suggests that the "elementary bodies" and "initial bodies" represent modifications of the organisms originally introduced which are brought about by the action of lytic substances contained in the conjunctival fluids and tissues.

The results of the work considered do not prove or disprove that the "elementary bodies," "initial bodies" and inclusion bodies are concerned etiologically in trachoma. This discussion is presented merely to show that these bodies in all probability are bacterial in character, and that the bacteria from which they originated are rod-shaped in form. The fact of their presence in a considerable percentage of cases of trachoma is an indication that they may be of etiologic significance, but the question cannot be answered definitely as yet.

AUTHOR'S SUMMARY.

RAT BITE FEVER. E. N. MCDERMOTT, Quart. J. Med. **83**:433, 1928.

In 1926, a case of rat bite fever was discovered in Edinburgh, and a strain of *Spirocheta morsus-muris* was isolated and its effects on animals investigated. One of the results was the analogy observed between the disease in certain animals and in syphilitic infection in man. Chill and fever may develop about thirty days after the bite has been received, but Japanese authors have claimed that the incubation period may be as long as two years. The wound heals rapidly; later pain is felt in the scar, and the surrounding area becomes swollen. Vesicles develop and ulceration ensues, the surface of which gives off a serous discharge, in which the organism is frequently demonstrable. Regional lymphadenitis follows. The temperature returns to normal, but an intermittent fever soon sets in. Malaise occurs with the attacks of fever, which is also accompanied generally by a maculopapillary eruption, which may be widespread. In long standing cases, a moderate degree of anemia develops. Occasionally, there are pleurisy and bronchitis, and in long-continued cases constipation is usual. The spleen is generally not involved. In the kidneys there is usually no involvement, except in severe cases, in which a toxic nephritis is common. Pains of the joints and periosteitis occur. The Wassermann reaction is positive in about 50 per cent of cases, and about 10 per cent of patients not treated die, but the mortality is negligible in the patients treated with neoarsphenamine. The posmortem observations may be summarized as follows: generalized marasmus; parenchymatous degeneration of the heart, liver and kidneys; catarrhal gastro-enteritis; catarrhal cystitis; meningeal hyperemia and edema; hyperplasia of the lymph glands; hyperemia of the suprarenal cortex, spleen and kidneys, and hyaline degeneration of the central arteries of the malpighian bodies in the spleen. In 1916, the causal spirillum was isolated by Japanese workers. Streptococci and bacilli were found in other cases, but these may be considered as secondary invaders. The strain studied by the author was isolated from a boy who was bitten on the forehead. About two weeks after this he was unwell, and a swelling developed; a few days later the regional lymph glands were enlarged. About a month after he received the bite he was feverish, and the brow and forehead were markedly swollen. He developed four attacks of high fever in the hospital, and a number of the cervical glands were excised on the forty-ninth day. The condition cleared up under treatment with neoarsphenamine. Emulsions of the glands were injected into two guinea-pigs, and spirilla appeared in their blood after nineteen and thirty-seven days. The organism is rapidly moving, refractile and stains deep violet with the Romanovsky stain; it is gram-negative, may be blunt or pointed, and may measure from 1.5 to 10 microns, with numerous flagella; it divides by transverse fissure and is extremely difficult to grow in laboratory mediums. Mice, rats, guinea-pigs, rabbits, cats and ferrets were injected, all of which developed lesions.

N. ENZER.

THE BACTERIOLOGY OF SPRUE. F. P. MACKIE, S. N. GORÉ and J. H. WADIA, Indian J. M. Research **16**:95, 1928.

The blood culture during life from thirty patients with sprue yielded an unknown bacillus in two. The rest were sterile to both bacteria and yeast. Protozoa such as amebas and flagellates are excessively rare in the feces in cases of sprue—probably as a result of their high acidity. Ninety-two specimens of feces from patients with sprue have been closely studied and a complete analysis of the last series of fifty-one cases is given in this paper. Not once in this series was a recognized pathogen isolated, though an atypical strain resembling *B. dysenteriae* (Flexner) was found once. *B. dysenteriae* (Strong) once, and organisms like *B. morgan, carolinus, columbiensis, asiaticus,* which are of doubtful pathogenicity, were each isolated on a few occasions. Hemolytic gas-producing anaerobes were not examined for in all cases but were invariably present when suitable cultures were made. As these organisms are almost invariably present in normal stools, the mere fact of their presence in stools in cases of sprue cannot in itself be claimed as significant. The duodenal contents of eight cases received particular attention. None was sterile. One case yielded five types of bacteria; one case, four; three cases, three, and two cases, two. A hemolytic streptothrix was present in two cases. Hemolytic strains of *B. coli* were not isolated, nor were anaerobes of types of *B. welchii* found in any of the eight samples. Half the strains of cocci were hemolytic, and a number of strains of hemolytic gram-positive aerobic bacilli were isolated from the duodenal contents. We may conclude that the duodenal contents from patients with sprue are rich in bacteria and that a number of these are powerful hemolytics. None of these could be definitely identified with the recognized pathogenic bacteria which are found in the intestinal canal in other diseases.

AUTHORS' SUMMARY.

COMPARATIVE INOCULATIONS OF BR. ABORTUS AND BR. MELITENSIS IN THE GOAT. E. BURNET, Arch. Inst. Pasteur de Tunis **17**:108, 1928.

The goat is susceptible to both the organisms which cause abortion in this animal. The susceptibility is somewhat more marked with respect to *Brucella melitensis.* It has been supposed that *B. abortus* may become virulent for man on passage through the goat, and the fact that undulant fever in man is acquired easily from the goat would support this idea. It is not possible to trace definitely the evolution of *B. abortus,* but it will be necessary in the further study of the epidemiology and prophylaxis of undulant fever to compare the pathogenic power of *B. abortus* for different species of animals.

THE ALBUMIN-GLOBULIN RATIO IN THE SERUM, SEDIMENTATION SPEED OF ERYTHROCYTES AND PIRQUET'S TEST IN PULMONARY TUBERCULOSIS. F. SCHEURLEN, Beitr. z. Klin. d. Tuberk. **69**:59, 1928.

The total protein content varies so much as to be clinically insignificant. The albumin-globulin ratio depends on the extent of the disease, the degree of destructive processes and the activity. Its determination may complete, but never substitute, a thorough clinical examination. The albumin-globulin ratio and the speed of sedimentation of the red cells are fairly parallel. They do not undergo any marked and definite alterations during a period of four months, while the Pirquet test increases in intensity in almost all active and nonprogressive cases.

MAX PINNER.

TUBERCLE BACILLI STAINED BY THE METHODS OF ZIEHL-NEELSEN AND MUCH. K. HAGEDORN, Beitr. z. Klin. d. Tuberk. **69**:166, 1928.

There are types of tubercle bacilli which cannot be demonstrated by Ziehl's stain, but only by Much's stain. A part of these take up the carbolfuchsin if they are first stained by Much's method. This property is retained if the bacilli are alternately stained according to the methods of Much and Ziehl.

MAX PINNER.

THE CULTIVATION OF TUBERCLE BACILLI OF LOW VIRULENCE, PARTICULARLY OF B. C. G., IN THE CORNEA. O. KIRCHNER, Beitr. z. Klin. d. Tuberk. **69**:181, 1928.

Tubercle bacilli of low virulence can be cultivated in serial passage in the cornea. This method excludes the possibility of spontaneous infection with another strain. This method will eventually permit the decision of whether or not the B. C. G. strain retains its low virulence in continuous animal passage.

MAX PINNER.

ANIMAL INOCULATION IN SURGICAL TUBERCULOSIS. M. KNORR and H. FRIEDRICH, Beitr. z. Klin. d. Tuberk. **69**:385, 1928.

Animal inoculation is the most reliable diagnostic method in surgical tuberculosis. It yields better results than the histologic examination of excised tissue. After intraglandular injection, the diagnosis could be made within four weeks in more than 85 per cent of cases.

MAX PINNER.

BARTONELLA ANEMIA OF RATS. V. SCHILLING and A. S. MARTIN, Klin. Wchnschr. **7**:1167, 1928.

The etiologic relationship of *Bartonella muris* (Mayer) was confirmed. Among thirteen splenectomized rats, two were found immune to splenectomy and inoculation. Of seven inoculated rats without splenectomy there were only four immune, the other three were infected with Bartonella blood or by cultures. Abundant growth was obtained once on blood agar, and the cultures were highly infectious. Six Bartonella rats, that spontaneously recovered after splenectomy, were reinfected either by blood transfer or by culture, and, accordingly, were not immune.

Morphologically and clinically, *Bartonella muris* differs markedly from *Bartonella bacilliformis* of Oroya fever and from the rod-like structures ("Erythrokonten Schillings") of pernicious anemia.

AUTHORS' SUMMARY.

MENINGITIS IN CHILDREN CAUSED BY KOCH-WEEKS BACILLI. H. MEYER and R. STEINERT, München. med. Wchnschr. **75**:945, 1928.

The symptoms, the clinical progress and the results of bacteriologic studies of three children with meningitis caused by Koch-Weeks bacilli are reported. None recovered, and the results of the postmortem examination of the brain of two are given. Both had a marked suppurative meningitis.

E. F. HIRSCH.

TETANUS BACILLI IN GANGRENE OF THE LUNGS WITHOUT TETANUS SYMPTOMS. W. VOIGT, München. med. Wchnschr. **75**:994, 1928.

Necrotic tissue from a gangrenous portion of the lung obtained by postmortem examination was examined in stained preparations and found to contain tetanus-like organisms. By cultures and animal tests, *B. tetani* was identified. The blood serum was not tested for tetanus antitoxin and it was not possible to explain the absence of symptoms of tetanus.

E. F. HIRSCH.

STOOL EXAMINATION OF 500 PHILIPPINE SCHOOL CHILDREN. L. LISSNER, München. med. Wchnschr. **75**:1163, 1928.

The total percentage of infestation by nematode parasites is 92.6. Of these, 16.4 per cent were *Anchylostoma duodenale*, 73 per cent *Ascaris lumbricoides*, 32.3 per cent *Trichocephalus dispar*, and 7.6 per cent *Oxyuris vermicularis*. An infestation with two varieties of parasites existed in 28.8 per cent. The ages of the children ranged between 8 and 18 years; those in the tenth to thirteenth years were most frequently infested. Unsanitary conditions of living are held responsible for the high percentage of infestation.

E. F. HIRSCH.

BACILLUS ABORTUS OF BANG AS A CAUSE OF A SEPTIC DISEASE IN HUMAN BEINGS. HORST HABS, Ztschr. f. klin. Med. **108**:445, 1928.

Bacillus abortus was described by Bang in 1897, and since was considered as being pathogenic only for animals. It is transmitted in cattle by the genital route and also by mouth when animals are fed on grounds soiled with this germ. Habs reports four cases in which the bacillus was isolated from the blood of patients in whom it caused a peculiar septicemia resembling somewhat that of undulant fever. The author is emphatic, however, that in these cases it concerned *Bacterium abortum* Bang and not *Bacterium melitensis* Bruce, basing his opinion on epidemiologic and serologic data. He also believes that the disease in human beings is apparently less rare than it is believed, being transmitted with the milk of cows. Four clinical histories of patients with this disease are given in detail, accompanied by a comprehensive discussion.

B. M. FRIED.

THE POTENTIAL ALKALESCENCE OF THE BLOOD AND ITS FLUCTUATIONS IN PULMONARY TUBERCULOSIS. T. D. KAHN, Ztschr. f. Tuberk. **50**:480, 1928.

The potential alkalescence of the blood in pulmonary tuberculosis is usually decreased. This decrease is roughly parallel with the extent and activity of the process. During exacerbations, hyperalkalosis is observed, followed by hypoalkalosis. Both rapid decrease and rapid increase in the alkalescence of the blood is prognostically an unfavorable sign.

MAX PINNER.

EXPERIENCES WITH THE SEDIMENTATION REACTION OF ERYTHROCYTES. H. STARCKE, Ztschr. f. Tuberk. **50**:486, 1928.

The prognostic and diagnostic evaluation of the sedimentation reaction must be made with great caution. The increase in the sedimentation rate, following a diagnostic injection of tuberculin, is not regular, it depends more on the dosage of the tuberculin than on the activity of the process. The fluctuations of the sedimentation rate do not give a reliable indication for tuberculin therapy.

MAX PINNER.

THE BLOOD PICTURE IN HUMAN TRICHINIASIS. W. BEHR, Folia haemat. **36**:25, 1928.

In four cases of trichiniasis, the blood showed constantly a high eosinophilic count. In two instances an eosinophilia of 14 and 11 per cent, respectively, was present in the blood three months after an apparent recovery.

B. M. FRIED.

SPONDYLITIS DUE TO BACTERIUM ABORTUS. L. P. JENSEN, Hospitalstid. **71**:637, 1928.

In Jensen's patient with an abscess in the left groin, a history of febrile disorder, diagnosed as typhoid a year earlier, and exposure to possible infection with *Bacterium abortum*, roentgen examination disclosed marked neoformations of the bones and destructive processes in the third and fourth lumbar vertebrae. Tuberculosis was excluded. While the roentgenographic observations resembled those in spondylitis typhosa, typhoid bacilli were not found at the time or on repeated tests since, but a definite serologic reaction to Bang's abortion bacillus was established after ten months of treatment.

BACTERIOPHAGES IN CHICKEN EMBRYOS. TAGE KEMP, Acta path. et microbiol. Scandinav. **5**:105, 1928.

A large number of publications deal with the search for bacteriophage lytic principles in embryonal organs of fowls and calves, and in human fetuses, though the writers have not succeeded in demonstrating them. In the experiments described in this paper, lytic principles were found in chicken embryos in a number

of cases. An attempt was first made to provoke the formation of lysins in a *Bacillus coli* strain by adding to the broth cultures of the strain varying quantities of coal-tar together with coal-tar and embryo chick tissue simultaneously; by these means, lysins were not formed in the *B. coli* cultures, but lysins were accidentally found in some of the chicken fetuses in the preparatory experiments for this series of tests. A number of embryo chicks were extracted from the eggs under sterile conditions and cut with scissors into 3 by 4 pieces; each piece was placed in a test tube containing common peptone broth and incubated for forty-eight hours at 37 C., without the appearance of any growth. A drop of *B. coli* culture was added to each tube, and, after twenty-four hours in the thermostat, the broth was filtered through a Seitz asbestos filter. Seven of the resulting filtrates contained active lytic principles against coli bacilli. Using special quantitative methods, which are described, the authors examined the relations of the seven lysins, when tested on colon, typhoid and dysentery strains, and proved that the lysins could be divided into three groups, showing outstanding differences in their action on the three strains of bacteria. Later information revealed the fact that the embryos from which the lysins had been isolated were derived from eggs from a poultry farm attacked by an epidemic of so-called white diarrhea; in a later examination of a large number of embryos (about 100) from eggs derived from healthy fowls, lysins could not be demonstrated in the embryos; it is therefore reasonable to connect the finding of lysins with the infectious disease prevailing in the poultry farm from which the eggs originated. It is difficult to bring these observations into harmony with the theory that the bacteriophage lytic principle is an ultramicroscopic virus.

AUTHOR'S SUMMARY.

THE ETIOLOGY OF ACTINOMYCOSIS IN DOMESTIC ANIMALS. H. MAGNUSSON, Acta. path. et microbiol. Scandinav. **5**:170, 1928.

A large number of cases of so-called actinomycosis in cattle and swine has been studied. Under actinomycosis are classed suppuration with granulation tissue in the pus of which are characteristic granules with a peripheral layer of clubs. Of the cases examined in cattle, 41 per cent were found to be due to streptothrix, and in the cases of actinomycosis of the udder of swine streptothrix was found in 66.3 per cent. In a number of cases, the causative organism appeared to be staphylococci. Actinomycosis should be regarded as a chronic inflammation due to certain pyogenic bacteria, having the power to adapt themselves and form cell colonies with their own intercellular substance and protective sheath and then live in symbiosis with their host. To Magrou, the naturalist, must be given the credit of having proved by comparative studies of botryomycosis and actinomycosis the identity of these diseases and of having given the best biologic explanation hitherto of the origin and character of the infection.

Immunology

ISO-AGGLUTININS IN THE NEW-BORN. C. H. SMITH, Am. J. Dis. Child. **36**:54, 1928.

It was observed, in 49.2 per cent of seventy-one cases of new-born infants, that the cord blood contained the full amount of receptors and iso-agglutinins so as to place it in one of the four designated groups. An increase in this percentage takes place during the first year of life.

An examination of the cord blood after an interval of ten days showed either a decrease in their titer or a complete disappearance in twenty-seven of the forty-one cases.

The iso-agglutinins of the cord and maternal blood have a qualitative similarity. Simple blood dilution does not seem to be the causative factor in the loss of iso-agglutinin strength following birth. The author is of the opinion that the similarity of iso-agglutinins of the child and mother and their subsequent diminution in the child following birth point to a transfer by way of the fetal cirulation.

The author cites two cases in which, after primary demonstration of iso-agglutinins, a subsequent disappearance occurred. Later the specific blood group of the infant was established by the appearance of an agglutinogen and associated iso-agglutinin. Thus the cord blood possessed both iso-agglutinins and could be placed in group O. The blood groups developed by the infant were A and B, respectively. Therefore, in establishing paternity by the iso-agglutinin reaction, it would be wise to wait until the permanent iso-agglutinins are developed.

The type of labor does not seem to have any effect on the placental transmission of iso-agglutinins.

H. E. LANDT.

ABSORPTION OF UNDIGESTED PROTEINS IN HUMAN BEINGS. H. SUSSMAN, A. DAVIDSON and M. WALZER, Arch. Int. Med. **42**:409, 1928.

Subjects used in this study were given passive local sensitization by the intradermal injection of serum from an egg-sensitive patient. An egg meal was given the following morning, and the time noted as to appearance of reaction in the sensitized area. Of thirty-four subjects tested, 85 per cent reacted positively, and 78 per cent of the reactions occurred in the first hour. With fish proteins previously tested, 86 per cent of the reactions occurred in the first thirty minutes. Giving the test protein on a full stomach showed the reaction time. Peptone feeding did not exert any consistent effect on the time or severity of the reaction. On rectal injection, absorption of the protein ocurred to a less extent than in the upper digestive tract.

HAMILTON R. FISHBACK.

PRODUCTION OF ACTIVE IMMUNITY AGAINST THE FATAL OUTCOME OF EXPERIMENTAL FECAL PERITONITIS. B. STEINBERG and H. GOLDBLATT, Arch. Int. Med. **42**:415, 1928.

Eleven dogs were immunized by repeated intraperitoneal injection of living colon bacilli. Ten of these survived the intraperitoneal injection of a dose of feces proved to be lethal for normal dogs. Of eight dogs immunized by the intraperitoneal injection of colon bacilli killed by heat, only three survived the normal lethal dose of feces. Feces heated to 60 C. for one hour proved sterile on culture, and did not produce peritonitis when injected into the peritoneal cavity of dogs.

HAMILTON R. FISHBACK.

SKIN SENSITIVITY OF RHEUMATIC SUBECTS TO STREPTOCOCCUS FILTRATES. E. I. M. IRVINE-JONES, Arch. Int. Med. **42**:784, 1928.

Fifty-six strains of streptococci were isolated from the upper respiratory tracts of rheumatic children, and twenty-four strains from nonrheumatic children. No significant difference in regard to source was found in fermentation, neutralization and skin tests on rheumatic and nonrheumatic children. Patients followed for a period of time showed alternation of positive and negative phases of the skin reaction similar to experimentally produced allergy. Some of these had recurrent rheumatic attacks. Skin reactivity was found to be most marked in rheumatic subjects, especially in the acute stages. Circulating toxins were not found in the blood of acutely sick rheumatic persons. It is suggested that rheumatic fever is a form of allergic response to common streptococci.

HAMILTON R. FISHBACK.

BLOOD GROUPING OF BAFFIN ISLAND ESKIMOS. PETER HEINBECKER and RUTH H. PAULI, J. Immunol. **15**:407, 1928.

The blood groupings of 166 Eskimos of Baffin Island are recorded. Further evidence is presented to support the view that the pure blooded Eskimo falls into blood group I.

AUTHORS' SUMMARY.

STUDIES ON THE ANTIGENIC PROPERTIES OF THE ULTRAVIRUSES. E. W. SCHULTZ and J. HOYT, J. Immunol. **15**:411, 1928.

No evidence of specific complement-fixing antibodies against the virus of herpes was found in the serum of rabbits which had been immunized by different routes with virus-brain suspensions. These immune serums also failed to present any evidence of specific precipitating antibodies. Virulicidal antibodies were demonstrable in all of the immune serums tested for this property.

AUTHORS' SUMMARY.

THE PRIMARY TOXICITY OF GOAT-SERUM. JOSEPH D. ARONSON, J. Immunol. **15**:465, 1928.

Fresh unheated normal goat-serum is hemolytic for guinea-pig erythrocytes; it is toxic when introduced into the general circulation or into the peritoneal cavity of normal guinea-pigs; it produces necrosis when injected into the dermis of normal guinea-pigs. The hemolytic, toxic and necrotizing action is dependent on a thermolabile and a thermostabile fraction. The thermolabile fraction reacts in the same way as complement. After it has been removed from goat-serum the addition of fresh complement restores the hemolytic and toxic action but not the necrotizing action. The thermostabile fraction is absorbed by certain tissues of the guinea-pig but not by the tissues of animals insusceptible to the action of goat-serum. Antigoat-serum neutralizes the hemolytic, toxic and necrotizing action of goat-serum. This neutralization is not brought about by complement fixation, but is brought about by the action of antibody on the antigen. Repeated injections of fresh goat-serum into normal guinea-pigs produce immunity to its necrotizing action, accompanied by the Arthus phenomenon. The appearance of the Arthus phenomenon is part of the process of immunity; it is not a result of the necrotizing action of the fresh goat-serum for it follows also injections of goat-serum from which the necrotizing property has been removed by heating. The hemolytic, toxic and necrotizing action of goat-serum is due to a single substance, which acts as a toxin.

AUTHOR'S SUMMARY.

IMMUNITY TO BOTULINUM TOXIN. MARY NEVIN and ELIZABETH LEE HAZEN, J. Immunol. **15**:489, 1928.

Rabbits can be immunized by injection, intradermal and intravenous, of antitoxin followed by toxin. Immunization by mouth or rectum did not succeed.

THE INCREASED SUSCEPTIBILITY OF THE ALBINO RAT INFECTED WITH THE TUBERCLE BACILLUS TO TUBERCULIN. M. I. SMITH, Pub. Health Rep. **43**:2817, 1928.

The object of this work was to ascertain the degree of increased susceptibility to tuberculin of rats infected with tubercle bacilli over normal animals, and the manner in which this is influenced by a diet deficient in vitamin A. The tuberculin preparation used was a water soluble powder suitable for intravenous injection, made from a synthetic nonprotein medium on which the bacilli had grown for six weeks. Rats on adequate and deficient diets were inoculated intraperitoneally with human tubercle bacilli, a procedure which has no appreciable effect on normal animals for several months. At varying intervals thereafter, the effect of the tuberculin preparation injected intravenously was determined. The minimum lethal dose for rats on the adequate diet was 300 mg. per kilogram for noninfected and 50 mg. per kilogram for infected animals. The ratio of susceptibility to tuberculin of the infected compared with the normal animal was therefore roughly 6 to 1. In animals suffering from vitamin A deficiency, the ratio was about 40 to 1, indicating that hypersensitiveness is increased sevenfold by the deficient diet. Hypersensitiveness was evident fourteen days after infection, and reached its height at thirty days. The same effect was produced by bovine as by human bacilli, but not by killed organisms.

It was concluded that invasion of the tissues of the rat by tubercle bacilli effects an increased susceptibility to tuberculin, as occurs in the more susceptible animals, and that the systemic effects of tuberculin are only quantitatively different in the tuberculous and the normal animal.

THE IN VITRO TITRATION OF B. WELCHII ANTITOXIN BY ITS ANTIHAEMOLYTIC POWER. J. H. MASON and A. T. GLENNY, J. Path. & Bact. **31**:629, 1928.

Identical results are obtained for *B. welchii* antitoxin by antihemolytic titration in vitro and by antitoxic titration in vivo by intravenous injection into mice.

AUTHORS' SUMMARY.

THE RELATION OF AGGLUTINATION BY SPECIFIC SERUM TO AGGLUTINATION BY ACID. J. A. ARKWRIGHT, J. Path. & Bact. **31**:665, 1928.

There are two agglutinable substances in a culture of motile bacteria of the *B. typhosus*, Salmonella group, which are responsible for agglutination by acid at different zones of hydrogen ion concentration or (H^+) and these substances are probably identical with the glagellar and somatic antigens, respectively, which react with H and O agglutinating serums.

THE ACTION OF IMMUNE SERUM ON VACCINIA AND VIRUS III IN VITRO. C. H. ANDREWES, J. Path. & Bact. **31**:671, 1928.

Immune serum does not destroy vaccinia virus in vitro, even after twenty-four hours' contact at room temperature. The inactivation of the virus is not associated with any stable antigen-antibody union occurring in vitro in twenty-four hours at room temperature. There is at present no evidence that any in vitro antigen-antibody union at all is involved. These conclusions are also applicable to virus III, though owing to greater difficulties in its study the evidence is less complete.

AUTHOR'S SUMMARY.

THE RELATIONSHIP BETWEEN THE BACTERICIDAL POWER OF NORMAL GUINEA-PIG SERUM AND COMPLEMENT ACTIVITY. JOHN GORDON and ARTHUR WORMALL, J. Path. & Bact. **31**:753, 1928.

The conclusion is reached that hemolytic complement and bactericidal complement have a similar constitution and are probably identical. Thus the bactericidal system investigated here appears to consist of an immune body (present in normal guinea-pig serum) and complement.

FIXATION OF COMPLEMENT BY B. TYPHOSUS AND NORMAL GUINEA-PIG'S SERUM. ERNEST M. DUNLOP, J. Path. & Bact. **31**:769, 1928.

Guinea-pig's serum shows zonal fixation of its hemolytic complement in the presence of a suspension in saline of the majority of strains of *B. typhosus*. Different strains of *B. typhosus* vary in their ability to cause zonal fixation of guinea-pig's complement. Two of twenty-one strains examined have failed to do so. The phenomenon is explicable on the basis of the presence in normal guinea-pig's serum of a natural antibody. This resembles immune antibody in its ability to sensitize the organisms, so that they can then fix complement, but it differs from immune antibody in its thermolability and in its comparative lack of specificity. This antibody varies greatly in its sensitiveness to heat and it is sometimes more labile than complement. *B. typhosus* unites with the antibody at 0 C., usually thereby becoming sensitized, but no fixation of complement occurs at 0 C. The antibody can be removed from guinea-pig's serum not only by organisms with which it reacts specifically (leading to complement fixation) but also by other organisms, and by agents such as coal dust charcoal or powdered glass. Hemolytic complement can be removed from guinea-pig's serum without

destruction of the antibody. Certain other organisms (coliform bacilli, anthracoid bacilli and staphylococci) also cause zonal fixation of guinea-pig's complement. The addition of heated normal serum (rabbit's or human) to mixtures of typhoid bacilli and guinea-pig's complement sometimes inhibits, sometimes increases, the zone of complement fixation.

AUTHOR'S SUMMARY.

REPORT OF THE ROYAL COMMISSION OF INQUIRY INTO FATALITIES FROM TOXIN-ANTITOXIN AT BUNDABERG. CANBERRA, M. J. Australia **2**:2 and 38, 1928.

Of twenty-one children injected with toxin-antitoxin on Jan. 27, 1928, some with the initial and some with the second dose, eighteen became ill and twelve died. The same bottle had been used several times previously without untoward effects. The symptoms were similar in all the fatal cases, beginning from five to eight hours after injection with vomiting and diarrhea, fever, rapid pulse, cyanosis and circulatory failure. The deaths occurred in from fifteen to thirty-four hours. The survivors developed abscesses at the site of inoculation. The most significant postmortem observation was degenerative change of the lymphoid tissue throughout, suggestive of the action of a toxic agent in the blood stream. Changes peculiar to the action of diphtheria toxin, such as fatty degeneration of the heart muscle or hemorrhages, were not found. Examination of the remaining toxin-antitoxin mixture showed it to be cloudy on the following day, and staphylococci were cultivated both from it and from the abscesses. No evidence of tetanus or of free toxin was found in the mixture, and injections of antitoxin in the sick children had no effect. The symptoms and pathologic observations pointed to a staphylococcus infection as the most likely cause of the illnesses, in spite of the rapidity of the deaths and absence of metastatic abscesses in the survivors. The organism was not highly pathogenic for laboratory animals, except for the rabbit. Skin tests in human beings with a filtrate produced marked reactions in some persons and none in others, suggesting a possible explanation of the failure of some of the children to react.

The batch of toxin-antitoxin in question was issued without addition of a preservative, and the bottle was kept at room temperature, so that conditions were favorable for the multiplication of organisms. There were two possible sources of contamination. The needles used for the injections were lifted out of sterile water with the fingers instead of with forceps, and air was necessarily injected onto the rubber-capped bottle as the fluid was removed. Although the fingers of the operator appeared to be the most likely source of the staphylococci in this case, an experiment was made to investigate the latter possibility. Air was injected and fluid removed with sterile needles in the usual manner from a number of rubber-capped bottles of toxin-antitoxin without antiseptic, and 30 per cent of them were found to be contaminated in this way. No contamination occurred in the presence of an antiseptic.

The commission concluded that the deaths were due to staphylococci with which the mixture was contaminated during the first injections made from the bottle, and that the repeated use of material from a rubber-capped bottle containing no antiseptic is dangerous. It recommended that biologic products in which growth is possible should not be issued in this form, and that all products not containing an antiseptic should be so labelled, with the warning that they must be completely used at one time or the remainder discarded. Special training for those engaging in the immunization branch of public health work was advised.

BEATRICE R. LOVETT.

LOCAL VACCINATION AGAINST RABIES. P. REMLINGER and J. BAILLY, Ann. de l'Inst. Pasteur **42**:349, 1928.

Using a series of guinea-pigs as test animals, attenuated rabies virus was locally inoculated by means of friction on the skin. Subsequent attempts to produce localized or general infection demonstrated a degree of immunity. The

practical value of strictly local immunization is discountenanced, but the doctrinal value in showing an affinity of the virus for the skin is upheld.

M. S. MARSHALL.

BLOOD GROUPS IN MAN AND CHIMPANZEE. JEAN TROISIER, Ann. de l'Inst. de Pasteur **42**:363, 1928.

The red blood cells of fourteen chimpanzees are agglutinated by human serums III (B) and IV (O); the human serums (AB) I and II (A) are without effect. The serum of the chimpanzees agglutinate human cells I (AB) and III (B). It is without effect on human cells II (A) and IV (O). The blood of chimpanzees possesses hemagglutinins (agglutinogen A and agglutin b) of group II of man. The quanity of agglutinogen and especially of agglutinins varies moderately in subjects, or from day to day in the same subject. The test of saturation of agglutinins does not enable one to distinguish the blood of man II (A) from the blood of the chimpanzees. Man II may receive with impunity intravenous injections of citrated chimpanzee blood. This humoral and cellular identity between the chimpanzee and man II favors the phylogenic hypothesis of a common ancestor.

AUTHOR'S CONCLUSIONS.

ALLERGY AND CLASSIFICATION OF PULMONARY TUBERCULOSIS. W. CURSCHMANN, Beitr. z. Klin. d. Tuberk. **69**:501, 1928.

The determining factor in the development of tuberculous lesions is the immunity and not the allergy. The immunity can be determined by tuberculin skin reactions with study of the blood picture. Positive allergy and absence of shifting to the left, with normal sedimentation time, indicates high immunity, while positive skin tests, with shifting to the left and increased sedimentation rate, indicate lowering of the immunity. It is necessary to indicate the gross anatomic character of the lesions such as, infiltration, dissemination, cavity, cirrhosis; type and extent of the lesion and its tendency toward progression, cavitation or retrogression. It is not necessary to create definite pictures of tuberculosis, and attempts at histologic classifications are futile and unnecessary.

MAX PINNER.

IMMUNIZATION OF GUINEA-PIGS WITH SAPONIFIED TUBERCLE BACILLI. K. W. CLAUBERG, Beitr. z. Klin. d. Tuberk. **69**:551, 1928.

Tubercle bacilli were saponified by 10 per cent potassium hydrate and exposure to heat. The final product was neutralized with lactic acid. Ten of twelve animals which were vaccinated with this preparation proved to be refractory to a subsequent infection; one developed some tuberculosis and one died of generalized tuberculosis. Four of the controls did not develop tuberculosis and five died of generalized tuberculosis three months before the completion of the experiment.

MAX PINNER.

INCREASE OF FLOCCULABILITY IN THE SERUM IN TUBERCULOSIS. J. VON DARÁNYI, Beitr. z. Klin. d. Tuberk. **69**:558, 1928.

This is a review of the literature dealing with the serum reaction known under the author's name.

MAX PINNER.

SPECIFIC-PROTEOLYTIC FERMENTS IN THE SERUM IN PULMONARY TUBERCULOSIS. F. LASCH and R. GROSS, Beitr. z. Klin. d. Tuberk. **69**:670, 1928.

Abderhalden's test was done on a small series of patients. The antigen was prepared from tubercle bacilli by boiling and extraction with lipoid solvents. Forty-six of sixty patients with pulmonary tuberculosis reacted positively. Three of fifteen healthy persons reacted positively. Pregnancy of not more than three months' standing did not interfere with the specificity of the reaction.

MAX PINNER.

THE VIRULENCE OF B. C. G. S. L. LANGE and K. W. CLAUBERG, Beitr. z. Klin. d. Tuberk. **70**:346, 1928.

The B.C.G. strain, even in massive doses, is but little virulent for guinea-pigs. The specific alterations which it produces remain localized and disappear again. The strain can be isolated from infected animals but neither the pure culture nor the infected organs produce any changes on reinjection into animals. The pathologic action of this strain could not be enhanced by submitting the animals to injuries, such as exposure to cold, inhalation of dust, vitamin deficient food, injection of sodium aurothiosulphate, injection of diphtheria toxin and mixed infection.

MAX PINNER.

THE COMPLEMENT FIXATION IN TUBERCULOSIS. M. STEIN and SCHACHSUWARLY, Beitr. z. Klin. d. Tuberk. **70**:408, 1928.

In a series of 136 patients complement fixation was done with the antigens of Wassermann, Klopstock and Neuberg and with an antigen of nonacid-fast organisms grown in saponin containing glycerol broth. The latter antigen gave the best results. But only 40.4 per cent of patients with clinical tuberculosis reacted positively, and 5 per cent of nontuberculous patients reacted positively.

MAX PINNER.

THE USE OF SERUM OBTAINED AFTER DEATH FOR ISO-AGGLUTINATION. P. SEREBRJANIKOFF and M. LEITSCHICK, Ztschr. f. d. ges. Gerichtl. Med. **12**:496, 1928.

Blood from the corpse gives good serum for iso-agglutination if obtained not later than seventy-two hours after death. Chronic diseases and injuries of the liver may be unfavorable for securing good serum. One drop of 20 per cent solution of formaldehyde to 2 cc. of serum is recommended as a preservative.

INVESTIGATIONS OF RELAPSE STRAINS OF TRYPANOSOMES BY MEANS OF THE RIECKENBERG PHENOMENON. F. LEUPOLD, Ztschr. f. Hyg. u. Infektionskrankh. **109**:144, 1928.

The Riekenberg phenomenon consists in agglutination of the blood platelets around the trypanosomes, when the blood of a mouse recovered from a trypanosome infection is mixed with blood containing organisms of an identical strain. No agglutination takes place with heterologous strains. The reaction has been shown to depend, not on the platelets themselves, but on antibodies present in the serum of the immune animals. The author found that immune serum could be injected into normal animals and their blood used for the reaction.

"Relapse strains" of trypanosomes were obtained by allowing mice infected with an original strain to recover partially (by giving insufficient doses of arsphenamine), and taking their blood during the subsequent relapse. Strains taken from different animals during their first relapse behaved like heterologous strains. Frequently no agglutination was observed when organisms from one animal were mixed with blood from another. It appeared, however, that the number of different strains obtainable from relapses was limited. Thus 56 per cent of the relapse organisms corresponded to one type, 20 per cent to a second, 20 per cent gave positive agglutination reactions with immune blood of both types, and 4 per cent with neither. The author concludes that the blood platelet reaction can be used in this way to identify and differentiate strains of trypanosomes. The results agreed with those obtained with the reinfection method, in which it is found that immune animals can be reinfected with heterologous but not with homologous strains.

B. R. LOVETT.

EXPERIMENTAL IMMUNIZATION WITH B.C.G. H. CHIARI, N. NOBEL and A. SOLE, Ztschr. f. Tuberk. **51**:354, 1928.

Following the administration of B.C.G. by mouth, guinea-pigs usually do not develop tuberculous alterations, probably because the bacilli are excreted with the feces. Pure subcultures from the original B.C.G. vaccine are virulent for guinea-pigs. Intraperitoneal immunization does not confer immunity to guinea-pigs. Most, but not all, of the treated animals lived longer than the controls after infection with fully virulent bovine bacilli.

MAX PINNER.

ATTEMPTS TO IMMUNIZE RABBITS WITH SYPHILITIC TESTICULAR TISSUE. M. HONDA, Acta Dermatologica **12**:179, 1928.

Male rabbits were injected in various ways with emulsions of rabbit testicles rich in virulent spirochetes, but no demonstrable evidences of immunity were obtained.

Tumors

PRIMARY NEOPLASMS OF THE HEART; REPORT OF AN UNUSUAL CASE. ERNEST B. BRADLEY and ELMER S. MAXWELL, J. A. M. A. **91**:1352, 1928.

The growth, which is regarded as primary in the heart, occurred in a man, aged 62. There were numerous metastases.

THE MALIGNANT FUNCTIONS OF THE CHORIONIC EPITHELIUM. W. BLAIR BELL, J. Obst. & Gynec. Brit. Emp. **35**:233, 1928.

Evidence is presented to show that the chorionic epithelium resembles malignant tissue of somatic origin in its function and chemical constitution. The high phosphatid-cholesterol ratio necessary for permeability of the cell membrane to water soluble foodstuffs and to the high water content of the cells is more pronounced in the trophoblast than in malignant tissue by its function of glycolysis in aerobic as well as in anaerobic conditions. There is a lower dextrose content and a higher lactic acid content in the placental circulation than in the normal venous circulation of the mother. This might indicate that, in regard to glycolysis, the chorionic epithelium functions like a malignant growth. Like malignant neoplasms, the chorionic epithelium shows a p_H value on the acid side of neutrality which is more pronounced in the younger placentas.

A. J. KOBAK.

MIXED TUMORS OF THE UTERUS AND VAGINA. WILFRED SHAW, J. Obst. & Gynec. Brit. Emp. **35**:498, 1928.

Mixed tumors of the female genitalia are classified according to their anatomic origin as follows: uterine, cervical and vaginal. Those arising from the body of the uterus are the rarest, there being only thirteen cases on record. The growth is polypoid and quite vascular, but its most dependent part is usually necrotic. The tumors develop after the menopause. Mixed tumors of the cervix occur at the average age of 34 and usually have a grapelike form. The mixed vaginal tumors are found in young children or infants and are usually polypoid. Mixed tumors are malignant and in association with sarcoma cells, they contain striated embryonic muscle cells, cartilage, fat, bone and elastic tissue. The author pointed out that the gelatinous matrix was similar to embryonal mesenchyme but, on the whole, he admitted that there is no satisfactory explanation of the origin of these tumors.

A. J. KOBAK.

ATTEMPT TO TRANSMIT CANCER OF THE HUMAN DIGESTIVE TRACT TO THE CHIMPANZEE. JEAN TROISIER AND HENRI LIMOUSIN, Ann. d l'Inst. Pasteur **42**:380, 1928.

The authors report that the results thus far are not successful.

THE HYPOPHYSIS AND DIENCEPHALON IN CARCINOMA. FRIEDRICH WOHLWILL, Deutsche Ztschr. f. Nervenh. **105**:63, 1928.

Only 5 of 177 cases of carcinoma showed metastasis in a hypophysis. Most of these were primary tumors of the breast. In 45 of 177 cases, instead of emaciation, a normal general physical condition or increase in weight were found. Discarding those cases in which the influence of other endocrine glands, notably the ovary, was suggested, the author accounted for eighteen cases on the bases of lesions in the hypophyseal-diencephalic vegetative centers.

CYTOCHROME IN TUMOR TISSUE. H. YACR, H. TAMIYA and W. NAKAHARA, Japan M. World **8**:233, 1928.

We examined four types of transplantable tumors for the occurrence of cytochrome, an intracellular respiratory pigment recently discovered by Keilin, and found that Fujinawa rat sarcoma contains a large amount, Flexner-Jobling rat carcinoma and Bashford mouse carcinoma a medium amount, and Rous chicken sarcoma either small or no demonstrable amount of this respiratory pigment. It is probable that these facts can be correlated with the difference in the ability of different types of tumors to withstand the deprivation of oxygen.

AUTHORS' SUMMARY.

Medicolegal Pathology

HUMAN MERCURIC CHLORIDE POISONING BY INTRAVENOUS INJECTION. E. L. HARMON, Am. J. Path. **4**:321, 1928.

The four patients in the cases here reported survived periods of intoxication ranging from six to twelve days following the intravenous injection of mercuric chloride in a dosage of more than 5 mg. per kilogram of body weight. Two types of necrosis were found in the kidneys in each of the four cases. The desquamation of renal tubular epithelium was not necessarily dependent on epithelial regeneration beneath the necrotic cells. Calcification was observed only in the epithelium, the seat of coagulation necrosis, and the calcified cells were seen in situ as well as free in the lumina of the tubules. In these cases the damage to the liver consisted of only a mild parenchymatous degeneration in contrast to the severe hepatic changes observed after mercury poisoning by mouth. Epithelial necrosis and mucosal ulceration of the colon were noted in the four cases. The renal changes due to mercury poisoning in man are essentially the same whether the mercury is administered by mouth or intravenously. Gastro-enteritis does not occur after the intravenous injection of large doses of mercury.

AUTHOR'S SUMMARY.

THE MIGRATION OF BULLETS, SHOT, AND NAILS AFTER INSERTION INTO THE VEINS OF DOGS. H. J. WARTHEN, JR., Arch. Surg. **15**:712, 1927.

Most of these metallic objects were carried along in the blood current through the heart into the lungs where they caused little trouble when sterile. The migration often required days. The experiments were made with fourteen dogs, and all but three of ninety-four of the foreign bodies introduced into the veins passed on into the lungs. These results in dogs are somewhat different from observations on the transportation of missiles in the veins in human beings, for as a rule after their arrival in the heart they usually remain there and are not forced out into the lungs.

E. R. LE COUNT.

TRAUMA OF THE CAUDAL REGION OF THE SPINE. H. KELLER, Internat. Clin. **2**:58, 1927.

With a good account of the relation of the coccyx to the many muscles and nerves of that region, seven cases of injury of the coccyx are reported: one with posterior dislocation, one with partial fracture from the sacrum and five with

sprains. Six of the patients were women, and one was a man. The pain at the time of injury is so severe that fainting is not uncommon. It radiates down the thighs and may extend up the trunk and even into the arms. Frequent urination and pain with defecation are symptoms. Much more aid to correct diagnosis is obtained by palpation than by roentgen examinations, for the coccyx is not bony. By conservative treatment recovery generally occurs. Laparotomy had been urged for two of the seven patients, and for two others removal of the coccyx. All recovered without operation.

E. R. Le Count.

Unsuspected Foreign Bodies in the Cranium. A. Fribourg-Blanc and H. Durand, Ann. de méd. lég. **8**:148, 1928.

Unsuspected Foreign Bodies. J. Belot, Ann. de méd. lég. **8**:132, 1928.

The enrichment of medical experience from great wars does not all take place during the period of conflict. It is certainly prolonged over the life of those who survive injury, and probably much longer. This fact is being demonstrated from time to time by opportunities to reexamine old injuries, by examinations made for the revision of pension awards, and by serious or fatal sequences which first appear years after injury. Two such late results, one concerned with an extensive trephining, the other with abrupt unexpected death probably from an abscess of the brain following injury requiring enucleation of one eye, were reported by Fribourg-Blanc (*Ann. de méd. lég.* **7**:52, 1927).

His more recent report with Durand of foreign bodies in the head is remarkable. It arose from systematic radioscopic examination of the heads of fifty exsoldiers in the neuropsychiatric service of the Military Hospital, Val-de-Grace, in Paris. Foreign bodies were found in the heads of thirty, and entirely unsuspectedly in all but one. These were intra-orbital, nineteen in the soft parts or bone and eight intracranial. In this last group there were six metallic foreign bodies.

Belot mentions a soldier with a missile in one buttock after it had passed through the trunk lengthwise from its entrance behind one shoulder as he was prone; a certificate possessed by another stated that he carried a piece of shrapnel in his right lung, but the fragment was found near the hip. In several persons the discovery of particles of glass was made some time after the wounds had healed. More interesting than these were intracardiac foreign bodies found in the hearts of three men, two seen during the war; all had entered from in front and lodged in the right ventricle. They were so violently in motion with the blood that considerable ingenuity was required to find them radioscopically; a timing exposure to the short intervals was necessary to photograph them. Examination of one of the soldiers after a year indicated a partial anchoring of the piece of metal. None of the three men was aware of the presence of these foreign bodies, nor were they greatly discommoded.

E. R. Le Count.

Experimental Venous Air Embolism. S. Frey, Arch. f. klin. Chir. **148**:536, 1927.

Probably the oldest of the theories explaining death from the entrance of air into the systemic veins is the failure of an adequate blood supply to the higher "vital" centers in the brain, because the air passes through the lungs into the systemic arteries. Another interpretation is distention of the right ventricle and auricle with froth so that blood is not forced through the lungs. A third is the failure of blood to pass through the lungs, with a resulting internal asphyxia, which Frey believes accounts for the death of the dogs used in his experiments. Air was put into the femoral veins, and what then happened depended largely on how much was injected and whether it was put in quickly or slowly. With considerable air introduced rapidly, there were large bubbles in the blood and a marked drop in arterial blood pressure. He found the pulmonary capillaries in dogs impermeable for air, but failed to observe any indications that cardiac paralysis occurred

because of acute dilatation. With a patient foramen ovale, air may easily get through into the left auricle and then into the systemic arteries. Frey found the air in the lungs of the dogs on roentgen examination, but not in the heart. He urges that by surgical measures the air be withdrawn from the pulmonary artery in cases of air embolism, aspirated possibly with a syringe.

E. R. Le Count.

Adipocere. S. Goy, Biochem. Ztschr. **187**:470, 1927.

The composition of adipocere from two bodies reported in 1922 is compared with the results of a chemical examination of this material recently obtained from a third body. In all of the material, the amount of free fatty acids was large; there was less olein than other fats. It is suggested that the low melting point of oleic acid may be connected with its disappearance.

E. R. Le Count.

Hemoglobin Resistance as an Index of the Age of Nurslings and Prematurely Born. H. Bischoff, Deutsche Ztschr. f. d. ges. gerichtl. Med. **11**:340, 1928.

When all are used together, the many conditions by which the duration of gestation of new-born infants is estimated are fairly reliable. Among these are the weight, length of the nails, consistency of the skin and size of certain foci of ossification. But these have little value for determining the age some weeks or months after birth at term, although by some investigators considerable reliance is placed on the length and size of certain bones. Bischoff has used the resistance of the hemoglobin to alkalis not only to ascertain the age of prematurely born infants, but also to decide during the first year of their life the time of conception of prematurely born infants, if the date of their birth is known. These are matters of importance in connection with foundlings, illegitimacy and questions of parenthood. The method was described by Korber in 1866 and has been somewhat modified since. Its details are too involved to be given here. The age is learned to within a few weeks. The tests were made by Bischoff on the blood of 100 nurslings and 38 prematurely born infants. Fetal hemoglobin is more resistant to the action of alkali than is the hemoglobin of adults, and that of a prematurely born infant is also more resistant than is the hemoglobin of an infant of the same age born at term. It is of considerable interest that these tests can be employed with the hemoglobin of decomposed or dried blood even though it has been kept for several years. The tests have been useful in demonstrating birth at term of infants already dwarfed.

E. R. Le Count.

Spontaneous Rupture of the Esophagus. Raestrup, Deutsche. Ztschr. f. d. ges. gerichtl. Med. **11**:373, 1928.

Explosive vomiting awakened a man early in the morning after a feast during which he ate considerable roast pig and sausage, and drank but little. The vomiting was followed by pain in the stomach radiating to the back. A diagnosis of perforated gastric ulcer was made, but when the abdomen was opened the stomach was found normal; death occurred about thirty-six hours after the feast. A tear on the right side of the cardiac end of the esophagus all the way through and 3 cm. long and also two smaller superficial tears of the lining were found after death; in the left pleural cavity, there were two liters of turbid fluid. No disease or anomaly of the esophagus was found to help explain the rupture.

Spontaneous rupture of the healthy esophagus is one of the rarest conditions responsible for sudden death. It usually is caused by violent explosive vomiting after excessive eating. Spontaneous rupture of the diseased esophagus may also occur. Attacks of coughing, collapse, dyspnea, sweating, restlessness and a rapid and weak pulse are the symptoms, as a rule, but in the case reported by Raestrup the symptoms were abdominal.

E. R. Le Count.

PUNCTURE OF THE SUPERIOR LONGITUDINAL SINUS OF THE DURA IN INFANTS. L. FABIAN, Monatschr. f. Kinderh. **36**:390, 1927.

Several deaths have resulted from needle wounds of the sinus or large cerebral veins made in the securing of blood or the administration of drugs intravenously, some from thrombosis and others from hemorrhage. Some deaths occurred because the patients were bleeders; others, because cerebral veins had been wounded accidentally and subdural hemorrhages produced. Some infants were atrophic and had myocardial degenerations. These conditions promote thrombosis in the dural sinuses where thrombosis is said to occur more easily than in other veins. Fabian believes that needle puncture of this sinus is dangerous and that indications for the procedure should be restricted.

E. R. LE COUNT.

THE ORIGIN OF PARALYSIS AGITANS IN TRAUMA. F. LOTMAR, Der Nervenarzt **1**:6, 1928.

That paralysis agitans may be caused by injury is a relatively modern conception, and apparently is steadily gaining ground. But the number of cases for which it is believed trauma was responsible is still small. Two cases reported by Hensegge (*Ztschr. f. d. ges. Neurol. u. Psychiat.* **110**:796, 1927) have been referred to in this section. There probably will be considerable interest in the account of two new cases by Lotmar, because the age of the patients was only 31 and 43 years, respectively, which is considerably earlier than the others previously observed. In the younger, the disease was not fully established until after a second fall, and in each the symptoms began within a few months after injury. The initial injuries were falls of from 11 to 14 feet. There is considerable difficulty in explaining how the paleostriatal changes peculiar to paralysis agitans in the aged and generally regarded as senile are produced by external violence, as a number of investigators have claimed that the changes in the basal ganglions are the same, whether the disease follows or has no possible connection with trauma.

E. R. LE COUNT.

FATAL CRIMINAL SUBACUTE POISONING WITH THALLIUM. L. KAPS, Wien. klin. Wchnschr. **40**:967, 1927.

A wife began to poison her husband in June by adding to his food "Zelio," a rat poison which owes its virtue to thallium sulphate. He came under observation in a Vienna hospital the next month where poisoning was suspected; improvement did not begin until he was denied food brought him from the outside. He was discharged on September 1, but returned in a much worse condition a week later and died, September 27. Although a medicolegal inquiry with postmortem and chemical examinations was made, it was not learned that death was caused by thallium until about two years later when the affair was reviewed in court.

About 30 Gm. of thallium sulphate was given at intervals of about three months. The symptoms were much like those due to lead and to chronic poisoning with arsenic when the symptoms result mainly from damage to the nerves. There were cramps in the stomach and abdomen, stomach distress, retching and vomiting. The joints of the toes and fingers were painful, but not swollen or red. The soles of the feet were sore, the ankles swollen and at one time articular rheumatism was diagnosed. An eruption of the skin of the eyelids and cheeks with superficial necrosis, ulcerative blepharitis and edema of the face developed. Total alopecia of the scalp occurred. It was diagnosed "toxic defluvium." The abdomen was generally tender, as were the muscles of the extremities, especially the peroneal groups. Hypnotics were required for restlessness at night, and shortly before death there was extensive suppuration in the cutaneous lesions of the face, and the patient became blind and demented. It was thought that retrobulbar neuritis caused the blindness.

Small traces of arsenic were found chemically after death in portions of the body taken for examination, but thallium was not looked for. Haberda presented a long review of the poisoning (*Beitr. z. gerichtl. Med.*, 1928, vol. 7). When thallium is added to the food of rats, it is asserted that calcium is removed from the bones and an alkalosis with hypochlorhydria or achlorhydria appears; also, disturbances of growth, testicular atrophy, changes in the lining of the stomach with the appearance of metaplastic stratified epithelium and hyperplasia and accumulation of heavily keratinized cells, alopecia and focal corneal opacities occur. It is also stated that children are less susceptible to thallium than adults. A few cases of acute poisoning in man with thallium have been reported. It is believed by Kaps that this is the first death from chronic poisoning with this rare metal. Thallium salts are chiefly used as depilatories. Organic compounds were first prepared a few years before this paper was written and have not yet gained extensive therapeutic use.

E. R. Le Count.

Miliary Tuberculosis and Trauma. F. Zollinger, Wien. med. Wchnschr. **77**:1281 and 1317, 1927.

It has been known for a long time that, in those already tuberculous, the disease may develop in an active form where tissues are injured, especially in bones. From such secondary lesions systemic infection by the blood, miliary tuberculosis, often has its origin. Zollinger believes that this last outcome of injuries is rare. Of 85,623 persons whose injuries were reviewed because of insurance, the question of tuberculosis arose in only 157. Miliary tuberculosis followed injury in one of these patients. From other sources, Zollinger states that trauma caused systemic dissemination in only 3 of 789 persons who were ill from tuberculosis, and in only 2 of 7,822 with injuries received during the war.

His review and bibliography are splendid, and many reports from the literature are aptly used. Among these are reports of deaths from miliary tuberculosis caused by injuries which crushed a tuberculous kidney and tore adhesions in the chest where tuberculous granulation tissue had bound the lung to the chest wall, and in a third person a tuberculous gland was torn by unusual straining. In another report cited, the cause was exposure to cold when the patient was wet. There must be a definite sequence of events in order to attribute the tuberculosis to injury with assurance. The earliest death from miliary tuberculosis has occurred fourteen days after the injury and the latest, eight weeks. The average time required for symptoms of generalization to appear is from ten to twelve days.

When miliary tuberculosis is evident only a few days after injury, the dissemination has preceded injury. The earliest recognition of miliary tuberculosis due to injury has been ten days. As a rule, it is difficult to maintain that trauma one or more years before played a part in causing miliary tuberculosis. It is highly important that hemorrhage in and about the injured tuberculous lesion be found at postmortem examinations and that the place be located where bacilli entered the blood stream and it should be decided whether that invasion would have occurred if an injury had not taken place. Miliary tuberculosis is frequently a sequence of influenza and measles, as is well known. That a profound blood poisoning which may be due to injury and infection with such bacteria as streptococci may also have generalized tuberculosis as its sequence is not commonly known to physicians. A general shaking up of the entire body or strains during which good muscular coordination is absent are more likely than are crushing injuries to cause tears of imperfectly organized vascular and edematous granulation tissue and result in an infection of the blood stream at that place with large numbers of tubercle bacilli, or to release the organisms so that intimal tubercles elsewhere are formed and serve as depots from which bacilli are discharged into the blood stream. The later is much more common, but at the place injured intimal tubercles in veins may develop or invasion of blood vessels by apposition-necrosis may result.

E. R. Le Count.

INTRACRANIAL LESIONS OR ASPHYXIA IN THE NEW-BORN. H. HEIDLER, Ztschr. f. Geburtsh. u. Gynäk. **91**:235, 1927.

This long review is almost wholly based on postmortem examinations, without, however, much reference to microscopic studies. There were six new-born infants who died from contusion of the brain, five from intracerebral, and five from intraventricular, hemorrhage. Hemorrhage into the leptomeninges was responsible for the death of twenty, and of these five were born dead. The most frequent change was a torn tentorium cerebelli, with bleeding under it from lacerated dural sinuses. Of the 131 infants, there was breech presentation in 65, forceps delivery in 37, spontaneous birth in 28 and, strangely enough, cesarean section in 1.

Death from asphyxia alone was relatively uncommon. Evidently, as a consequence of careful postmortem examinations, asphyxia as a cause of death of the new-born infant will be of less importance than it has been heretofore. The examination of the cranium and its content is most important.

E. R. LE COUNT.

THE RELATION BETWEEN INJURY AND TUBERCULOSIS OF THE TESTIS AND EPIDIDYMIS. F. ZOLLINGER, Ztschr. f. Tuberk. **48**:119, 1927.

It is likely that most of the statements in medical literature about the frequency with which tuberculosis of the testes and epididymis is caused by injury are without proper foundation. For example, in a report of the health of the Prussian army, from 1900 to 1905, of 112 cases, 73 were attributed to injury; in another report by Peter, 22 of 117. Zollinger believes that such ratios are altogether too high. Of 85,623 accidents dealt with officially in the cantons of Aargau and Solothurn in Switzerland, where he is engaged in compensation work for insurance companies, only 157 reports dealt with the question of tuberculosis, and in only three of these was tuberculosis of the testicle and epididymis mentioned. In these, inquiry failed to substantiate the alleged relation to trauma.

Details of these three, of eight hitherto unreported cases and of five from the literature are reviewed. In order satisfactorily to predicate a causal relationship to injury, the circumstances of the injury should be undeniable and there should be symptoms such as swelling of the testicle, subcutaneous hemorrhage and pain. The frequent accounts of "strain" are unimportant, and the tuberculosis does not follow open wounds. Failure of the person to stop working at the time of injury is a good indication that the alleged trauma is without significance. Indirect violence, such as falling and lighting on the feet, which may result in a concussion of the testis or epididymis, is important, but with this exception the violence must occur directly to the parts. The injury must be sufficient to promote localization of bacilli at the wounded place or to loosen tubercle bacilli already mobilized there.

In the last event the tuberculosis antedates the injury, and symptoms of an aggravated active tuberculosis soon begin. When as a consequence of injury bacilli locate in previously healthy epididymis or testicles, the disease should not begin until three or four weeks and not later than from two to three months following the injury. When the consequences of injury merge gradually into outspoken tuberculosis of these parts with such proper time relations and without an interval of entire freedom from symptoms, trauma is relevant. In the spontaneous development without trauma of tuberculosis of these male organs, the disease usually spreads from the prostate gland to the epididymis, and from there to the testis. Prostatic tuberculosis is usually (70 per cent) a sequence of pulmonary lesions.

E. R. LE COUNT.

NEPHROLITHIASIS AFTER FRACTURED SPINE. E. SIMON, Ztschr. f. Urol. **21**:444, 1927.

One third of the total amount possible was decided as proper indemnification in the Free State of Danzig for the loss of a kidney removed for stones due to an injury. This was to be paid for three years, because by that time the other kidney would function normally. The injury was fracture of the spine in the lumbar region with paralysis of the lower extremities, which disappeared about four weeks after laminectomy.

About four months after the injury there were symptoms of pyelitis of the right side, and one month later, a diagnosis of stone was made. The kidney was removed six months after the injury; the patient recovered. Blood was found in the urine soon after the fall. The upper pole of the kidney removed had a healed injury in it with blood from an old hemorrhage. The stones were not of the kind, heavily phosphates, which result from inflammation, and the upper pole of the right kidney where the injury occurred was at the level of the transverse processes of the first lumbar vertebra, which were fractured. These were reasons for the decision that the nephrolithiasis was caused by the injury.

Calcium carbonate with a little phosphate was found in one concretion examined chemically. A trace of blood was also demonstrable. Other stones in the kidney resulting from injury have been largely oxylates. Whether or not calcium from the several broken vertebrae and from bones of the lower extremities, which were paralyzed for several weeks, contributed to the formation of stones is also discussed.

E. R. Le Count.

Injury of the Rectum and Urinary Bladder by Radium and X-Rays. L. Zeiss, Ztschr. f. Urol. **21**:626, 1927.

There are repeated observations of ulcers, gangrene, and fistulous passages of the urinary bladder and rectum from the use of radium and roentgen-rays which kill remaining tumor cells and prevent recurrences after operations for cancer of the adjacent organs. In women these have followed cancer of the uterine cervix especially; in men, cancer of both prostate and bladder. These irradiation lesions are particularly interesting because they have repeatedly been taken for recurrent tumors. After he had found such masses in the bladder, one surgeon planted the ureters in the front wall of the abdomen; later, when he was ready to remove the entire bladder, he found the tumor absent and the bladder lining smooth and healthy. The lesions are frequently raised, thick, solid masses with broad bases and surfaces beset with warty elevations. The surfaces may be ulcerated and are often heavily coated with urinary concretions. They are high up in the bladder as a rule, and may be as much as 3 or 4 cm. in diameter. About the masses, the mucosa is usually edematous, and bullae may be present.

The calculous masses change position and obstruct the urethra, so that it is necessary to crush them repeatedly and remove them in fragments. Tissue removed from these supposed tumors by curetting has been found to be composed solely of an inflammatory exudate and thrombosed blood vessels, both necrotic in places. They are extremely chronic and heal very slowly.

In the three patients with such localized lesions in the bladder reported by Zeiss, all were first noticed months after the radiation and all were at first regarded as recurrences of tumors. Some ulceration was still present in one patient four years after the radium was used. In this patient, a circular stenosis of the rectum also resulted from the treatment; it was firm and fixed, and was first diagnosed as recurrent carcinoma, but it disappeared spontaneously.

E. R. Le Count.

Technical

The Volume of the Blood. D. C. Darrow and T. E. Buckman, Am. J. Dis. Child. **36**:78, 1928.

A rapid electrometric method for determining the freezing point of small quantities of fluid is described.

Authors' Summary.

The Cerebrospinal Fluid of Premature Infants. J. Glaser, Am. J. Dis. Child. **36**:195, 1928.

The most frequent cause of unsuccessful lumbar punctures in premature infants is the pushing forward of the loosely attached dura of the posterior wall of the spinal canal by the entering needle. Unsuccessful lumbar punctures in premature

infants will occur much less frequently if the tap is made with the child sitting in an upright position and when a fine hypodermic needle is used. If the proper technic is used, lumbar puncture in these infants is a relatively safe procedure and is often of great value in the diagnosis of and the treatment for cerebral hemorrhage. Bloody spinal fluid obtained from these infants is suggestive but not diagnostic of intracranial hemorrhage. Blood due to the trauma of the puncture frequently cannot be differentiated from blood present as the result of intracranial hemorrhage. The presence of red blood cells in microscopic numbers in the spinal fluid of premature infants during the early days of life is a physiologic phenomenon. The most common form of intracranial hemorrhage in premature infants is subpial hemorrhage. Fatal cerebral hemorrhages may occur in premature infants without the appearance of gross blood or even an exceptional number of microscopic red blood cells in the spinal fluid. Xanthochromia is a physiologic phenomenon in premature infants during at least the first four weeks of life. The degree of xanthochromia of the cerebrospinal fluid may be conveniently measured quantitatively by means of the icteric index. The icteric index of the cerebrospinal fluid of premature infants is highest during the second week of life. More than half of the yellow spinal fluids of infants give a positive indirect van den Bergh reaction. Yellow spinal fluids occur with a relatively high icterus index, and give neither a positive benzidine test nor a positive van den Bergh reaction. The color of these fluids is presumably due neither to hemoglobin nor to bilirubin but probably to a pigment or pigments intermediate in composition between these two. A positive direct van den Bergh reaction of the spinal fluid is strongly suggestive that the patient has had an intracranial hemorrhage. It may, however, occur in kern icterus without cerebral hemorrhage. Cerebral hemorrhage probably contributes to the degree of icterus neonatorum, and unrecognized cerebral hemorrhage is perhaps the cause of some cases of so-called "physiologic" icterus neonatorum.

H. E. LANDT.

A MODIFIED HALDANE GAS-ANALYZER FOR USE WITH SMALL VOLUMES OF GAS. H. C. BAZETT, Am. J. Physiol. **86**:556, 1928.

A modified gas analyzer is described which is capable of analyzing mixtures of carbon dioxide and oxygen with a neutral gas in any proportion, in volumes up to 2 cc. The error varies from 0.04 to 0.2 per cent according to the volume of gas available.

H. E. EGGERS.

THE SPINAL FLUID TEST OF TAKATA AND ARA. B. L. MONIAS, J. Lab. & Clin. Med. **14**:67, 1928.

The Takata-Ara test is based on the formation of colloidal mercuric oxide when mercuric chloride and sodium carbonate or sodium hydroxide are added to normal spinal fluid. Fuchsin serves as an indicator and stains the fluid a bluish violet. Pathologic spinal fluids either produce a precipitation of the mercury with absorption of the dye or a change of the color to purple or pink. The flocculation is characteristic of syphilis, while the change of the color is observed in bacterial meningitis. The test has given satisfactory results in more than 100 spinal fluids and compares favorably with the colloidal gold test. In tabes dorsalis, the test is less sensitive than the colloidal gold test. The following solutions are required:

A 0.3 per cent solution of sodium chloride.

A 10 per cent solution of sodium carbonate. This solution is recommended by Takata and Ara but can be replaced by a tenth normal sodium hydroxide solution of which the double amount is taken. In cases of meningitis, the change in color is more distinct with the sodium hydroxide than with the carbonate.

A 0.5 per cent solution of mercuric chloride.

A 0.02 per cent solution of diamond fuchsin in distilled water.

The spinal fluid must be free from blood and should not be kept longer than twenty-four hours before making the test.

A series of ten standard Wassermann test tubes are set up, and 1 cc. of the 0.3 per cent sodium chloride solution is placed in the second tube and up to the tenth tube. One cubic centimeter of the spinal fluid is placed in the first and second tubes and after mixing, 1 cc. is withdrawn from the second tube and filled in the third tube; this procedure is repeated with the following eight tubes. The last cubic centimeter of the dilution is discarded. There is now a range in the dilution from the concentrated spinal fluid in the first tube to 1.512 in the last tube. One drop from a capillary pipet of the sodium carbonate or two drops of the sodium hydroxide solution are added to each tube and mixed well. The test is completed by the addition of the Takata-Ara reagent. This reagent consists of equal parts of the mercuric chloride and fuchsin solutions mixed immediately before using. The solutions of mercuric chloride and fuchsin keep indefinitely. Three-tenths cubic centimeter is used in each tube. The test tubes are shaken thoroughly and kept at room temperature. Takata and Ara first recommended three readings, namely, after fifteen minutes, thirty minutes and twenty-four hours. Monias believes that some information can be obtained after thirty minutes and that the result becomes final after twenty-four hours. As stated, two different forms of reactions may take place with pathologic spinal fluids.

S. A. Levinson.

Further Observations on the Urobilinogen Test for Liver Function. G. B. Wallace and J. S. Diamond, Contributions to Med. Science Dedicated to A. S. Warthin, George Wahr, Publisher, Ann Arbor, Mich., 1927, pp. 407-413.

In a large number of normal persons and patients suffering from disease in which the liver was not involved, Wallace and Diamond found the concentration of urobilinogen in the urine to be remarkably constant. In the normal person the urobilinogen content of the urine is below that amount which gives a positive color reaction with Ehrlich's benzaldehyde reagent when the urine is diluted 25 times or more. The age, the time and quality of the food intake, constipation, excretion of indican and the time of collection of the specimen do not influence the reaction.

Wallace and Diamond have produced experimental damage to the liver parenchyma and have induced a measurable increase in excretion of urobilinogen. Parenchymatous disease of the liver, as seen clinically, was accompanied by a similar increase. The main factor in the production of excessive urobilinogen may be an abnormally large formation of urobilinogen in the intestine, but probably in every instance high urobilinogen excretion is an evidence of abnormal function of the liver.

Walter M. Simpson.

A New Gold Sol for the Lange Test. Frank Scott Lowweather, Brit. J. Exper. Path. **9**:161, 1928.

A modification of the Mellanby and Davies method of procedure for the colloidal gold test is described, in which a double bromide salt is used instead of gold chloride. By this method, more uniform results are obtained.

Pearl Zeek.

A New Method for the Preparation of Bacteriological Culture Media Containing Agar. A. R. Grace, Australian J. Exper. Biol. & M. S. **4**: 269, 1927.

Two pieces of fine gauze are cut about 1 foot square. From a roll of cotton-wool a piece about 8 or 9 inches square and a few millimeters thick is carefully removed, taking care that there are no thin patches, and is laid between the pieces of gauze. The center is then pushed down into a liter Erlenmeyer flask, and the required amount of agar, in a granular condition, is introduced. The pad is then formed into a bag, with the agar in the center, and tied firmly with string at the mouth. The excess gauze is cut off and the bag suspended in the flask by means of strings which are conveniently held by a rubber band around the neck of the flask.

Seven hundred and fifty cubic centimeters of distilled water is poured into the flask and the bag is allowed to float loosely in the water. It is then autoclaved for twenty-five minutes at 120 C., allowed to cool to 100 C., and removed from the autoclave. The bag is raised up toward the neck of the flask clear of the liquid and replaced in the autoclave for ten minutes at 110 C. to allow all the agar to drain from the bag. When cool, the empty bag is removed from the flask, leaving the clear agar jelly. The reaction may then be adjusted and the nutrient broth, filtered and adjusted to the same reaction, added.

T. Brailsford Robertson.

Examination of the Cellular Constituents of the Spinal Fluid. E. Forster, München. med. Wchnschr. **75**:1877, 1928.

After centrifugation, the sediment is mixed with a small drop of serum and the mixture divided into three portions on separate slides. The first portion is spread widely and dried in the incubator; the second is spread thin like a drop of blood, and the third is mixed with methyl-green pyronin and examined under a cover glass. After drying and methyl-alcohol fixation, the first and second preparations are also stained with methyl-green pyronin.

Edwin F. Hirsch.

Society Transactions

THE PHILADELPHIA PATHOLOGICAL SOCIETY

Stated Meeting, Oct. 11, 1928

J. HAROLD AUSTIN, *Presiding*

CARCINOMA OF ABERRANT THYROID (CARD SPECIMEN). E. L. ELIASON.

W. M., aged 20, in 1921 was operated on for incision and drainage of a cervical abscess. The microscopic report on the node removed was tuberculosis and, by a second pathologist, normal tissue. In 1927, three nodes were removed from the same site and, after much discussion, diagnosed as carcinoma of an aberrant thyroid. In 1928, another node was removed and regarded as benign adenoma by some and as adenocarcinoma by others.

The section of the node excised in 1921 showed a practically normal cervical node, without evidence of tuberculosis or of the tumor which formed subsequently at the same site.

The node excised in 1927 showed a rather thick fibrous capsule from which thin strands projected into the interior to form the framework of branching papillae. These were covered with a layer of columnar cells. A few of these cells showed mitotic figures. In several places there were small pink-staining masses which might be either colloid or serum, probably the latter. Scattered through the section were deposits of calcium. The diagnosis was papillary cystadenoma of unknown origin, either thyroid or parotid.

The node removed in 1928 showed a preponderance of epithelial cells and little interstitial tissue. Many blood vessels were seen in the supporting framework. In places the cells were in papillary formation, while in others they were in definite acinar formation containing colloid. In most areas the cells were of the low columnar type in single layers, resting on fibrous tissue. There was variation in the size and formation of the acini. In a few areas, the cellular arrangement was indefinite and incomplete. Some of the acini showed an intra-acinar papillary proliferation of the epithelial cells like that seen in hyperplastic toxic goiter. On account of the location of the tissue, the acinar formation with colloid and the immature cells in areas, it seemed that this was an aberrant thyroid undergoing malignant changes.

SARCOMA OF AN INTRA-ABDOMINAL UNDESCENDED TESTICLE. MOSES BEHREND.

J. F., aged 29, complained of a swelling in the right iliac fossa. In March, 1927, he consulted a physician, who told him he had an undescended testis and a growth in the abdomen. The swelling did not disturb him until a year later, at which time he consulted another physician who advised operation. The patient said that the mass was getting larger and painful. Operation in March, 1928, revealed a large tumor attached to the cord and lying free in the abdominal cavity. The inguinal canal was not disturbed. The mass measured 14 by 10 by 6.5 cm. One surface was white and smooth; others appeared fleshy and partially lobulated. Section of the tissue showed the entire absence of normal glandular structure, and in its place was a mass of round cells with deeply staining nuclei. A moderate fibrous stroma was present. The diagnosis was small round cell sarcoma.

MUCOCELE OF THE APPENDIX (CARD SPECIMEN). E. L. ELIASON.

A. C., a man, aged 50, complained of pains in the back. These were found to be due to hypertrophic arthritis (x-rays). During the examination, a large mass was found in the abdomen. The patient was not aware of its existence. It was the size of an orange and of the "phantom tumor" type in that it could be made to disappear, reappearing later at a different site. There were no suggestive

abdominal symptoms. The specimen presented was a mucocele of the appendix. The microscopic section showed the fibrous coat of the appendix greatly hypertrophied. Between the layers of this were areas of lymphocytic infiltration and calcium deposits. All that remained of the epithelial lining of the appendix were the few cells which were seen in the lumen.

AMEBIC COLITIS. REPORT OF TWO CASES. GEORGE M. ROBSON.

Because the author believes that the occurrence of a severe and rapidly fatal type of amebic colitis in this part of the country is of interest, he reported two cases. The first patient was from Philadelphia, the second from Delaware. Unfortunately, no fuller record of their residences and travels is available.

The first case was that of a white man, aged 46 years. During the summer of 1927, following some dietary indiscretion, he developed diarrhea which continued intermittently for several months. This subsided during the fall only to reoccur late in December. He was admitted to the service of Dr. Stengel at the University Hospital in February. At that time he was running a septic temperature and was weak, pale and emaciated. He became progressively worse and died in March with evidence of a generalized peritonitis. During his stay in the hospital, he had had several severe hemorrhages from the bowels.

The autopsy revealed a generalized acute seropurulent peritonitis. The small intestines were free from lesions, but the whole mucous surface of the colon was covered by numerous ulcers, varying from a few millimeters to several centimeters in diameter. These were acute with soft purulent bases and reddened hemorrhagic margins. In most places the entire wall was thickened, soft and inflamed. Many of the ulcers penetrated deeply into the muscular layers. Just proximal to the splenic flexure there was a large gangrenous area involving almost the entire circumference and measuring 6 cm. in length. Here the intestinal wall had sloughed away, its contents failing to escape into the general peritoneal cavity only because of adhesions to the omentum and transverse mesocolon. The main perforation was on the posterior surface and here the necrotizing process had involved a portion of the pancreas and come almost into direct contact with the portal vein. This vessel was thrombosed at this point and from there into the liver it was filled with pus. The liver was riddled with pylephlebitic abscesses.

Microscopic examination showed suppurative and necrotizing ulcerations of the colon with many amebas in the lesions. Amebas were present deep in the muscular layers of the intestine. The liver showed only a suppurative process, no amebas being found in it. Cultures and smears from the peritoneal exudate, pus from the portal vein and from the abscesses of the liver showed streptococci and colon bacilli.

The second patient, a white man, aged 51, was admitted to the service of Dr. Frazier at the University Hospital in April, 1928, complaining of paralysis of the right hand. A syphilitic lesion was suspected, and the patient received some mercurial preparation. Following this, on May 1, diarrhea and fever developed, which were at first regarded as being caused by the mercury. He became progressively worse, with a septic fever and abdominal distention; finally fecal vomiting occurred and a silent abdomen. He died in June.

At autopsy an acute generalized peritonitis was found. The colon including the rectum was the site of innumerable ulcers varying from a few millimeters to a few centimeters in diameter. This process was limited to the large intestine, the small intestine being free from ulcerations. Some of the ulcers were superficial, but many were deep, eroding and undermining ones. Their margins and bases were soft and covered by a purulent exudate. In the cecum and sigmoid the process was especially marked with large interstitial abscesses, and a thickened, friable wall. In these regions the serosa was covered by a plastic exudate. One large branch of the portal vein within the liver was filled by a septic thrombus, but the process was still confined to the vessel and its wall. The portion of the liver supplied by this vessel was sharply demarcated from the rest of the liver by its dark red color which contrasted sharply with the lighter yellowish-red of the rest of the organ.

Sections from the colon showed a suppurative and necrotizing process with great numbers of amebas in all layers of the wall. Amebas were also seen in several small blood vessels; none were found in the liver. The cerebral lesion was found to be due to a thrombotic process. Bacteriologic examination showed a mixture of streptococci and colon bacilli in the peritoneal exudate, in the pus from the interstitial abscesses and in the material from the portal vein.

The cases are similar in the excessively extensive and acutely ulcerative colitis, and in the presence of a bacterial peritonitis and pylephlebitis. The acute and suppurative character of the lesions of the intestines suggest that bacterial invaders were responsible for at least part of the damage there. It seems evident, from the lack of amebas and the presence of bacteria in the lesions of the peritoneum, portal vein and liver, that these lesions were secondary to the bacterial infection of the amebic ulcers. One is tempted to surmise that the sequence of events in these two cases may have been as follows: There was in each case a quiescent amebic infestation which was rather suddenly incited to acuteness, in one case by diet and in the other by medication, in this way opening a portal for the entry of bacteria which thus gained access to the peritoneum and portal vein and caused peritonitis, pylephlebitis and abscesses of the liver.

LEUKOSARCOMA WITH A PRIMARY RETROPERITONEAL TUMOR AND TERMINAL BLOOD INVOLVEMENT. D. H. FLASHMAN and S. S. LEOPOLD.

A white man, aged 60, gave a history of a swelling in the right inguinal region for twelve months. Biopsy showed lymphosarcoma. A roentgenogram revealed a tumor in the pelvis. The leukocyte count was normal. He was given roentgen treatment for from four to five months, during which time several examinations of the blood gave negative results. In the following month he developed leukemia, the white blood count rapidly increasing to 444,000 cells per cubic millimeter at the time of death. The differential blood count showed from 90 to 96 per cent small lymphocytes. The autopsy revealed a primary, invasive lymphosarcoma in the inguinal and retroperitoneal regions, an extensive involvement resembling leukemia of most of the lymphoid system, liver, spleen and bone marrow of the right femur, and metastatic nodules in most of the organs. In the tissues there were both large and small lymphoid cells, with intermediate types and in various combinations, indicating that these cells represent different degrees of hyperplasia or differentiation. The picture appeared intermediate in character between typical lymphosarcoma and lymphatic leukemia, rather than a combination of two separate entities. The case falls in the group of leukosarcoma.

THE RELATION OF VITAMIN B TO INFECTION AND IMMUNITY WITH SPECIAL REFERENCE TO B. WELCHII. WILLIAM B. ROSE.

During the course of an investigation in which dogs were fed a diet deficient with respect to vitamin B, one animal developed severe convulsions. As is well known, such neuromuscular manifestations are typical of vitamin B deficiency. In this particular case, death seemed imminent. The only chance to save the animal's life was the immediate parenteral injection of a vitamin B concentrate (Cowgill, George R.: Am. J. Physiol. **66**:164, 1923). Accordingly, vitamin powder (Harris) was dissolved, neutralized and sterilized by boiling for three minutes, and the solution was injected subcutaneously. Practically all the symptoms were alleviated within a few hours.

A few days later, however, a mass developed at the site of the injection. Palpation revealed gas crepitus. It was then recalled that the material injected had not been sterilized sufficiently to destroy resistant spores. A provisional diagnosis of "gas infection" was therefore made. The abscess was incised and drained and a pure culture of *Bacillus welchii* isolated. The animal was fed large doses of material rich in vitamin B and recovered rapidly. Repeated cultures subsequently made from the site of the wound proved to be negative for *B. welchii*.

Immunologists agree that dogs are naturally immune to *B. welchii*. However, this dog suffered from vitamin B deficiency when infection occurred, and recovered

rapidly when this vitamin was administered. These considerations suggested the possibility that this vitamin may play a rôle in the maintenance of natural immunity to this organism in dogs.

It should be stated that the diet employed in these experiments was composed of an artificial mixture of isolated food substances. Consequently, it was deemed advisable to determine whether or not dogs possess a natural immunity to *B. welchii* under these conditions of experimentation. Accordingly, a series of dogs fed an artificial diet which was complete in every known respect were inoculated with *B. welchii*. No untoward symptoms were observed. The same dogs were then deprived of vitamin B, and positive blood cultures were obtained. Vitamin B therapy was then instituted, and the blood cultures became negative. This procedure was repeated a number of times, and the same results were obtained.

These observations suggest that a lack of vitamin B in the diet of dogs lowers their resistance to infection with *B. welchii*. However, in view of the fact that dogs suffering from "acute" vitamin B deficiency manifest anorexia, loss in weight and in the advanced stages neuromuscular disturbances, it is obviously unsafe to conclude that the increased susceptibility to infection was due to a lack of vitamin B per se. It was therefore deemed essential to rule out these factors before any precise interpretation could be placed on the results.

Accordingly, dogs were given just sufficient vitamin B to maintain a perfect appetite for the artificial ration. In some instances, there was an actual gain in body weight; the animals remained active and apparently normal. Furthermore, to eliminate the possible effect of confinement on their resistance to infection, they were permitted to run about in an open courtyard and bask in the sunshine. In spite of these favorable conditions of experimentation, positive blood cultures were obtained. The bacteremia was not accompanied by fever or other symptoms. After the administration of vitamin B therapy, the blood cultures were invariably negative. It would appear, therefore, that a state of chronic vitamin B deficiency favors the growth of *B. welchii* in the blood stream of dogs.

Another aspect of this subject was investigated. Five dogs, apparently normal, were inoculated with *B. welchii*. Massive injections were made subcutaneously, intraperitoneally and directly into the circulation. Blood cultures remained negative for months.

The possibility of a latent infection was considered. After five months these dogs were deprived of vitamin B. In two of these dogs the blood cultures were still negative. These animals finally developed convulsions and died from vitamin B deficiency; postmortem cultures of the internal organs were negative.

Three animals lived and yielded positive blood cultures which became negative when vitamin B therapy was instituted. Hence, it would appear that natural immunity is not a constant phenomenon in this species. Whether or not this variability in natural immunity is directly related to the vitamin reserve of these animals remains to be investigated. The fundamental factors which control the immunity of dogs to this bacterial organism is not clear. It seems, however, that a lack of vitamin B breaks down this natural immunity.

In general, the results appear to indicate that nutritional factors deserve consideration in immunologic studies and that vitamin B in particular merits attention.

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Oct. 11, 1928

HARRISON S. MARTLAND, *Presiding*

THE DETAILED DIFFERENTIATION OF BACTERIA BY MEANS OF THE MIXTURE OF ACID AND BASIC DYE AT DIFFERENT p_H. A. V. TOLSTOOUHOV.

As was reported previously, methylene blue-eosin Y mixture can be used for differential staining of bacteria. It gives a fairly deep-staining of bacteria at p_H about 3 and above. Below p_H 3 the eosin Y stains the bacteria only a pale

pink. At this low p_H the eosin Y is present mostly as an undissociated eosinic acid, and for this reason there is not enough eosin ions to stain bacteria. To improve it eosin was replaced by a stronger acid dye—acid fuchsin. The mixture, methylene blue (methylthionine chloride, U. S. P.), medicinal, Merck and acid fuchsin can be used successfully at p_H as low as 0.8. The method of staining by this new mixture is entirely the same as with the old mixture of methylene blue-eosin Y. It is sensitive enough to detect the difference in the iso-electric points: (1) of single bacteria from the same pure culture; (2) of different strains of *Bacillus coli* and *Bacillus typhosus*. The new mixture can also be used for the study of chemical composition of the different parts of the bacterial body. Applying it at a certain p_H it is possible to stain differentially polar bodies of the typhoid group and *Bacillus diphtheriae*. These new mixtures are recommended for the staining of *B. diphtheriae* as a substitute for Neisser's stain. It is interesting to note that polar bodies of the colon group consist of more alkaline protein than the body of the bacterium itself; they are stained by acid fuchsin. The polar bodies of the bacteria, on the contrary, are composed of more acid protein than the bacterial body; they are stained by methylene blue.

As a result of this study, the precipitation of the dyes depends on the degrees of hydrolysis of the formed salt, dye and protein of the tissue or other dye. If there is salt, weak base and comparatively strong acid (as for example, nucleo-proteins or phosphatides above their iso-electric points), the degree of hydrolysis at the same dilution will be the highest in the case of the salt formed with the weakest acid. As is known, the gram-positive bacteria are composed of a stronger acid ampholyte than the gram-negative organisms.

Combining these facts, one should expect that basic dyes form almost insoluble salts with gram-positive bacteria; on the contrary, the solubility of the salt formed from basic dye and gram-negative bacteria will be much higher.

From this purely chemical standpoint the different bacteriostatic effects of basic dyes on the gram-positive and gram-negative bacteria are explained.

DISCUSSION

ALFRED PLAUT: I am not a chemist, but I want to bring before you practical experience from the histologic laboratory; this experience I am sure you all have had from time to time. So much has been said about acid and alkali, and the combining of the acid dye with the alkaline tissue, and vice versa. I have had the experience when only one slide of an interesting material had been stained with hematoxylin that without doing anything else but bringing it back into water one could stain the same slide for elastic tissue by the Weigert stain. Any one can do this with a slide stained previously with hematoxylin or another dye. Paraffin sections which were stained first with pyronin-methyl green and later stained with Pappenheim's panoptic stain gave excellent results, occasionally better ones than with the unstained slide. The point I do not see, is how these tissues give the same chemical reaction after having reacted chemically with the first dye. Furthermore, when tissue is decalcified, it goes through strong acids, then weak alkalis and then water; or when a piece of tissue has been left on the table and dried out, it is put into antiformin, in which the hard material becomes soft again, and still good results are obtained with the staining. How is it that material which has been chemically ill treated in such a way gives the same acid-alkaline reaction as it gave before?

HARRISON MARTLAND: Is it possible to stain living or dead bacteria with colloidal solutions or are not most of the stains used ionic in character? I had the impression from Blair Bell's work that he was trying to get lead into the cancer cell in colloidal form. It was my experience that such colloidal solutions must change into ionic forms before they could penetrate the cell membrane except in such cells as the phagocytic histiocytes of the reticulo-endothelial system.

A. V. TOLSTOOUHOV: In reply to Dr. Plaut's question, I can say that from the chemical standpoint the different compounds used in histologic technic should be divided into three groups:

(1) Chemically moderately active compounds, like picric acid, potassium bichromate, mercuric chloride and formaldehyde, which form stable, practically insoluble salts with tissue protein. For this reason they almost permanently change the iso-electric points of the tissue proteins or, in other words, their staining properties.

(2) Chemically weak compounds like dyes, which form more soluble salts with tissue protein than the compounds of the first group. Besides that, the solubility of these salts depends on the reaction (p_H) of surrounding mediums. On account of these properties the dyes cannot permanently change the iso-electric points of tissue protein; in other words, the tissues stained by one dye can be stained by another dye without any effect from the previous stain.

(3) Chemically active compounds, like strong mineral acids or strong alkalis. They form a well-ionized salt with tissue proteins, and they cannot change the iso-electric points of tissue protein. The presence of these compounds in smallest quantities in staining mixtures changes effectively the p_H of the mixtures. In this instance, the p_H of a mixture and not the compounds themselves change the staining properties of the tissues. The detailed discussion of this subject can be found in my previous articles, delivered before the society in 1926 and 1927.

In reply to Dr. Martland's questions, I can say that the dyes often form colloidal solutions, and still they can penetrate the cell membranes. If one considers the toxic effect of a substance, it is not necessary for the dye to penetrate into the cell. To kill the cell it is only necessary to form precipitate on the cell membrane and make the membrane impermeable for food supply or products of the cell metabolism.

THE IMPORTANCE OF THE VESSELS IN THE ROUND LIGAMENT TO THE HEAD OF THE FEMUR DURING THE PERIOD OF GROWTH AND THEIR POSSIBLE RELATIONSHIP TO PERTHES' DISEASE. A. P. ZEMANSKY, JR., and R. K. LIPPMANN.

Despite an extensive literature concerning the vessels in the round ligament, little accord has been reached concerning their importance to the femoral head. Recent studies of the pathology of Perthes' disease have strengthened the plausibility that it is primarily an aspetic subchondral necrosis. The possibility that this disease may be directly due to occlusion of the round ligament vessels is thus deserving of further consideration, and has suggested the following study, namely, an effort to determine more accurately whether these vessels are normally of notable importance to the femoral head, especially during adolescence.

A preliminary series of dissections in rabbits showed that the developmental stage of the capital epiphysis in animals, 2 weeks old, corresponds approximately to that of children, 4 years of age. These dissections further demonstrated that, in these animals, the femoral head unites with the shaft at about the age of 7 weeks (18 years in the human being). With regard to this epiphysis, then, the span of life between the ages of 2 and 7 weeks in these animals corresponds roughly to that between 4 and 18 years in the human being, the age period during which Perthes' disease occurs.

A subsequent series of arterial injections, according to the method of Dr. Gross, demonstrated that, during this time, there is an artery which courses through the round ligament and enters the femoral head. This artery, however, becomes smaller in size as the animal grows older and an injection could not be made after the age of 7 weeks—approximately the age at which the epiphysis unites with the shaft.

With these facts in mind, we have sectioned the ligamentum teres on one side in a series of rabbits 2 weeks old, thereby obliterating the circulation through the ligament to the femoral head in order to determine the effect of this procedure on the developing capital nucleus. The rabbits were killed at intervals thereafter, and subjected to examination. As control, a similar operation was performed on the opposite hip of the rabbits, but the ligament was not cut. Changes in the

femoral head did not ensue. Also, three adult animals were operated on in a similar manner; the femoral of these animals were not affected.

The femoral head changes which followed the section of the round ligament in rabbits 2 weeks old may be classified as follows:

1. Anemia. Anemia of the anterior portion and crest of the nucleus was observed first in the femoral heads of the two rabbits killed on the sixth day. Anemia of this area characterized also all of the subsequent specimens observed.

2. Signs of Bone Necrosis. Pyknosis and failure of the bone cells to stain in the anemic area were first observed in the nine day specimen. The number of the bone cells thus affected increased in the later specimens up to the 22 day old rabbit in which practically the entire anemic area contained only empty cell lacunae.

3. Marrow Necrosis. Necrobiosis was observed to occur in the marrow cells of the six day specimens. Failure of the hemogenic elements to stain and incipient marrow fibrosis were first apparent in the nine day specimen. Necrosis of the marrow stroma was first observed in the twenty-two day specimen.

4. Signs of Cessation of Ossification in This Area. Grossly, the relatively smaller size of the bony nucleus was first apparent in the eighteen day specimen. The thickening of the surrounding cartilage and the increased proportion of unossified cartilage in the bony lamellae of the nucleus could be seen microscopically in the same specimen and in all those subsequently observed. The diminution in number of the osteoblasts was first apparent in the nine day specimen. In the last three specimens, osteoblasts were not identifiable in the affected area.

5. Gross Deformation of the Femoral Head. Flattening of the weight-bearing area and ridging occurred first in the nine day specimen. The later specimens show in addition, pitting and furrowing of the surface. Microscopically, the cartilage of this area is well stained and intact.

6. Coxa vara. First apparent in the eighteen day specimen and present in all the subsequent ones.

The fact that these changes were, in all the specimens, accompanied by anemia, and the resemblance of these changes to those of infarction, suggest strongly that they were due to the circulatory interference of the operation. When it is considered that the affected area is, at this age period, directly supplied by the vessels of the ligamentum teres (as our injections have shown), and that the changes fail to appear when the operation is performed after the vessels have closed, there can remain little doubt that the pathologic picture results from obliteration of the round ligament circulation alone.

It may thus be concluded from these experiments that the vessels of the round ligament are essential, at least in rabbits, for the normal development of the femoral head, and that interference to the circulation through the vessels at an early age produces an anemia of the weight-bearing portion of the capital nucleus, which, in turn, causes bone and marrow necrosis with ensuing secondary deforming changes. These changes are strikingly similar to those of real Perthes' disease.

Furthermore, our studies have demonstrated that, as adolescence progresses, the importance of these vessels gradually diminishes until the epiphysis unites with the shaft, at which time, in normal animals, the vessels no longer carry blood into the femur and the nutrition of the crest is derived entirely from below.

DISCUSSION

P. WILLIAM NATHAN: I am not sure whether the gentlemen here are interested in the clinical side of Perthes' disease. We have had few opportunities of examining actual specimens in Perthes' disease in the various stages of its development; the specimens we get are all probably from the later stages of the disease and show late terminal changes. I have seen the slides from Dr. Lippmann's experiments. There is no doubt that necrosis of the growing epiphysis occurs after the ligamentum teres is cut, indicating that the blood supply through the ligamentum teres is important to the growing femoral head. The question of

its relationship to Perthes' disease may be another matter, but I am convinced that cutting the ligamentum teres actually results in a necrosis of the spongiosa, and that these changes are similar to those found in specimens from so-called Perthes' disease. It must be admitted, however, that there are additional phenomena to be found in these specimens, namely, changes indicative of an inflammatory reaction as evidenced by the presence of granulation tissue. It must also be admitted that there are some necrotic changes as the result of what is called aseptic necrosis. I think the aseptic part of it is somewhat of a misnomer, because one sees similar changes as the result of streptococcus or other nonsuppurative infection of the joints. I believe that most authors, when they speak of aseptic necrosis, mean simple bone necrosis unattended by suppuration. Axenhausen believes that the majority of these cases are due to trauma which involves the vascular supply and leads to these changes. I think that Dr. Zemansky's and Dr. Lippmann's work will throw light on the condition of Perthes' disease. The disease is one of the growing period, so that it may well be that some trauma that affects the ligamentum teres leads to occlusion of the vessels, and in consequence the changes demonstrated by Dr. Lippmann this evening.

HARRISON MARTLAND: Is coxa vara a prominent feature of Perthes' disease?

P. WILLIAM NATHAN: There are various malformations and some resemble coxa vara. In former years coxa vara was considered a disease by itself, and supposed to be due to some softening process. We also find the condition known as slipping epiphysis in adolescent boys and girls, and especially in boys who have some disturbance in growth, generally fat boys with a delayed development of the genital organs, the so-called pituitary type; these children are particularly liable to slipped epiphysis, and according to the x-rays, the process in the bone is somewhat similar to the changes in Perthes' disease. We do have cases in which the epiphysis is displaced, but the majority of cases reported as Perthes' disease show softening of the head and bending, and biopsy specimens show destruction of the subchondral spongiosa without any actual microscopic change in the joint. In some there are changes in the epiphyseal disk which remind one somewhat of the changes seen in rickets, though they are obviously not in any way related to rickets. In these children there seems to be a grave disturbance of these structures, not alone in the head of the femur, but in the other spongy bones, a condition which is now called an epiphysitis. We now see cases in which necrotic areas are found in the tarsus, scaphoids and inflamed epiphyses or tibial tubercle. All these cases occur in children, and they will hinder or interfere with the development of the epiphysis. The subject is still not well understood, and in older children these conditions that appear during the course of development seem to have something to do with imbalance of glandular secretion, because all these cases occur about the age of puberty.

HARRISON MARTLAND: I was interested in the occurrence of coxa vara. In cases of occupational poisoning by the ingestion of radioactive luminous paint in the watch dial industry, we found a coxa vara present in one of the fatal cases and in two cases in which the patients are still alive. This was attributed to a radiation osteitis of the head of the femur due to the deposition of fixed deposits of radioactive substances in the bones. The marrow was replaced by a loose, fibroblastic and myxomatous tissue similar to that which has been reported as due to mechanical cutting off of the blood supply. Any softening of the femur in this region, no matter what lesion causes it, is likely to cause a coxa vara due to the added weight of the body.

R. K. LIPPMANN: I did not expect to take up the clinical application. At present it is impossible to say whether or not we have reproduced true Perthes' disease. We have shown, however, that there must be a balance which exists in the adolescent femoral head between the vessels which cross through the round ligament and those which supply the head from the side, because if the vessels from the round ligament are cut off, a certain part of the head necroses. At a later date this is not true. This balance which probably exists in the human being as

well may have some connection with the endocrinal disturbance that has been noted in 90 per cent of the cases of true Perthes' disease.

We probably do not have any right to call the necrosis of Perthes' disease an aseptic necrosis; however, we do not have any evidence of infection in the majority of the examined specimens of the true disease.

AN INSTANCE OF DIFFUSE ENDARTERITIS OBLITERANS. DAVID PERLA and B. SELIGMAN.

A woman, aged 40, with a history of repeated miscarriages, developed, four years before admission to the hospital, neurologic symptoms, loss of ability to read, write and understand spoken language and left-sided hemiplegia. She subsequently complained of severe substernal pain, gangrene of two toes and hemorrhagic sputum. The Wassermann test was 4 plus. A clinical diagnosis of cerebral spinal syphilis had been made. She died suddenly of acute purulent leptomeningitis.

At autopsy a diffuse endarteritis obliterans of medium size and small arteries was found in the spleen, uterus, ovaries, kidneys, brain, lungs and heart. There was an old infarct of the left ventricle, healed infarcts in the spleen and brain, a marked arteriosclerosis of the kidneys, a complete obliteration of the inferior vena cava and organized thrombosis from the level of the renal veins to the iliac vein.

Sections of the vessels showed a proliferative process of the intima almost entirely with part or complete occlusion of the vessels involved. There was no evidence of inflammation, blood pigment indicative of thrombosis, or syphilis, and spirochetes were not found.

The etiology of this peculiar case is unknown, and only one similar instance was found in the literature. The possible cause was discussed.

DISCUSSION

JAMES EWING: I am greatly interested in this remarkable case. I was impressed by the thoroughness of the report, and the soundness of the reasoning which the authors have employed. I would suggest for their consideration that possibly the exaggerated extent of the physiologic processes of involution such as occurs in the vessels of the uterus might account for the result. One cannot fail to see the humor of the speaker in his remark that in spite of the 4 plus Wassermann reaction the patient had signs of syphilis. Were there any other signs of syphilis, such as a smooth tongue, flat scars in the aorta, pigmented spots in the skin or any other of the old fashioned indications of syphilis? Also how many hours did you spend hunting for spirochetes in these tissues? The lesion did not impress me as easily explainable as syphilis.

HARRISON MARTLAND: Did the intracardiac thrombus involve the right ventricle?

DAVID PERLA: No, it was entirely on the left.

HARRISON MARTLAND: I suppose some of the secondary terminal infarction might be due to the intracardiac thrombus.

DAVID PERLA: No, there was not any evidence of any blood pigment.

We looked with unusual thoroughness for spirochetes, and at the time of autopsy we looked for evidence of syphilis. The diagnosis was made on the basis of the 4 plus Wassermann reaction, the numerous miscarriages and the wide involvement of the central nervous system which could not be explained on any other basis. In a woman 40 years of age it seemed justifiable to make such a diagnosis. But, as the spinal fluid did not show indication of syphilis, by either the Wassermann reaction or the mastic test, I feel convinced that this is not a case of syphilis. We made Levaditi stains of many of the vessels.

JAMES EWING: Did I understand that there was one other instance of this condition in the literature?

DAVID PERLA: I found a reference to a case of Bronsom in a boy, aged 12, who had an obliterative process in the extremities, brain and coronary vessels of the heart; this was briefly reported in 1915. He was unable to explain them on the basis of syphilis. In addition, some one has told me that Vollhard casually mentions a universal endarteritis obliterans as a cause of hypertension, but I have been unable to verify that reference.

BASAL CELL CARCINOMA, ADENOID CYSTIC TYPE: THREE CASES WITH GENERALIZED METASTASES. JOHN W. SPIES.

For convenience and clarity, the paper is divided into three parts, and in each a distinction is made between tumors having cutaneous origin and those arising in noncutaneous areas.

1. Resumé of the literature. An attempt is made to show the gradual change in point of view. The first reports dealt with a somewhat benign process; later ones demonstrated the local malignant condition of some of these lesions, and even the capacity to metastasize to regional lymph nodes (basal cell carcinomas). Rodent ulcer and adenoid cystic epithelioma were often confused with one another. In tumors arising from noncutaneous areas, a distinction must be made between adenoid cystic basal cell carcinoma and adenocarcinoma.

2. The analysis of cases from the General Memorial Hospital. The need of early and effective treatment is emphasized. Roentgen therapy is the method of choice. When surgery is employed, it should usually be as a secondary adjunct. This conclusion is drawn on account of the comparative radiosensitivity in this group of tumors and the fact that surgical failures are numerous. The mortality in the cutaneous group is low, but it is high in the noncutaneous group. The latter result seems to be explained in the majority of the cases by the advanced stage of the disease (usually due to inadequate treatment), and to the anatomic relations of the palate, nose, accessory nasal sinuses, orbit and cranial cavity. It is from these locations that most of the noncutaneous tumors arise, or into which they extend.

It is the opinion of the author that none of the cases from the Memorial Hospital definitely belong to the cutaneous disease known as adenoid cystic epithelioma (Brooke), which may be characterized by a slow course, familial tendency and multiple tumors (usually nonulcerating and benign). Our cases exhibit adenoid and cystic features, but are more closely related to the usual type of basal cell carcinoma (Krompecher), which may be distinguished by more rapid progress, nonhereditary qualities and single growths (usually broken down and malignant).

A detailed description of the histologic structure is given.

3. Case reports. In this disease an important point to be noted in the loss of life that may occur in those patients in whom the disorder remains uncontrolled over a long period of time. This is definitely shown by three cases with generalized metastases. In these, one was primary in the skin near the right nasolabial fold; one arose from the mucous membrane of the nasal septum, and one originated in the submaxillary salivary gland, or the skin adjacent to it.

DISCUSSION

JAMES EWING: Dr. Spies has properly emphasized the considerable clinical importance of the fact which these patients demonstrate, that basal cell carcinoma of this type is not the local and confined disease which we had thought it to be, and not one which can be dealt with by temporizing measures. I myself was closely concerned with two of these cases, and when metastases developed in the lungs and the spine, I was greatly surprised. In fact, in both instances, I felt sure from what I had read and seen of the disease before that no such events could be expected. That error has been corrected in the last edition of my book "Neoplastic Diseases," in which it is stated that this tumor may produce widespread metastases. As for the actual identity of all the tumors, it is not necessary to claim that. The cutaneous group and the glandular group from the histologic

standpoint do not show points of essential difference. It is probable that in both instances the cells of origin have similar tendencies which they show in different degrees in recurrences and local and general metastases. The tumors of the tongue and lung are different looking, but of the same general type, and from twelve to fifteen years later the tumor has not greatly changed. The whole experience has taught a new lesson in the natural history of this group of epidermoid carcinomas. I suppose that the reason why this phase of the natural history of adenoid cystic carcinoma has not been emphasized before lies in the fact that under exclusive surgical treatment the patients died before metastases developed or became obvious, whereas under roentgen therapy the local disease is controlled for many years so that the metastases have time to develop and assert themselves.

DEMONSTRATION OF A SERIES OF HEARTS ILLUSTRATING SUDDEN DEATH IN CARDIAC SYPHILIS. HARRISON S. MARTLAND.

Dr. Martland presented a series of hearts illustrating the various phases of cardiac syphilis. They were taken from cases of sudden deaths.

Concerning the anatomic route through which the aorta was infected he favored the mediastinal theory of Klotz. In the secondary stage of syphilis, as the stage of spirochetemia passes away, the various organs attempt to rid themselves of the organisms by way of the lymphatics. The important lymph node groups in the mediastinum, especially those around the bifurcation of the bronchi and trachea, become collecting stations or reservoirs for the storage of drained spirochetes. There, they are exposed to the phagocytic action of the histiocytes of the reticulo-endothelial system. Most authorities have for years agreed that the earliest histologic lesions in syphilis involving the aortic root will be found around the vasa vasorum in the adventitia, where there is a mantel of lymphocytes and histiocytes with an obliterating endarteritis. Numerous *treponema* can often be found in these areas. It is, therefore, reasonable to suppose that the lesion in early aortic syphilis is suggestive of lymphatic extension from the reservoirs in the mediastinal lymph nodes by retrograde lymph flow into the perivascular spaces around the vasa vasorum, and that the obliterating endarteritis and formation of miliary gummas occur afterward in the nature of a defense reaction. To support this supposition there were abundant pathologic and clinical observations calling attention to the surrounding mediastinitis and periaortitis. The mediastinal involvement can often be recognized by roentgenographic examinations. The clinical symptoms in aneurysm, especially the pain, are often relieved by antisyphilitic treatment causing a melting away of the mediastinitis, while the physical signs of aneurysm, such as size, pulsation and murmurs, may be intensified by the relief from the surrounding tense barriers.

His experience has led him to believe that the main and most important lesion in nearly all cases of cardiac syphilis is a supravalvular sclerosis of the aortic root on which most of the other changes and phases of cardiac syphilis depend. The earliest lesions are microscopic and occur around the vasa vasorum in the adventitia of the root of the aorta in which there is seen a collection of lymphocytes and histiocytes in the perivascular lymph spaces. Stained sections of these areas may show numerous spirochetes which have gained access to this area by a retrograde lymphogenous route from the reservoirs of spirochetes in the peribronchial and mediastinal nodes. Small miliary gummas are formed in the adventitia with characteristic histiocytic giant cells, necrosis, etc. A secondary invasion of the mediums follows, with consequent breaking up of elastic tissue and weakening of the vessel wall.

The lesion has now developed to a stage in which it can be recognized grossly. The earliest patch is often a triangular area situated just distal to the mutual attachment of the aortic cusps, in the so-called commissures. The base of the triangle is usually pointed distally. The first patch is often seen in the commissure nearest the orifice of the left coronary artery. The patches are gray, or slightly yellowish, elevated with sharp edges, smooth on top, or marked by shallow

furrows separating trivial secondary elevations. Later, these patches become more or less confluent, and the process spreads in a horizontal manner around the root of the aorta. In his opinion, the sinuses of Valsalva, contrary to most authorities, frequently escape. The syphilitic sclerosis is often extensive and extends over the entire arch to the great vessels of the neck, and sometimes into the descending aorta. Frequently in the decrescent period of cardiovascular life the syphilitic sclerosis is almost entirely masked by a superimposed arteriosclerotic lesion, and in these cases a correct interpretation was always difficult.

The danger of supravalvular sclerosis is three-fold. In about 60 per cent of the cases the process invaded the aortic cusps by way of the commissures, producing aortic regurgitation. The commissures were widened, the free edges of the cusps thickened, the cusps retracted and wrinkled, and marked hypertrophy and dilatation of the left ventricle ensued. Aortic regurgitation is the most malignant type of cardiac syphilis. Patients rarely survive two years after the original diagnosis is made, and intensive treatment is dangerous and of little use.

In about 30 per cent of the cases the supravalvular sclerosis encroached on the orifices of the coronary arteries and produced a narrowing or atresia. This was more apt to occur when the orifices of these arteries were congenitally high placed above the base line; this occurred in many persons. When the orifices had their origin in the sinuses they frequently escaped. Such a narrowing or atresia might account for sudden death with or without pain, or angina and occasionally coronary occlusion indistinguishable from that produced by arteriosclerosis. Real coronary occlusion in his opinion, however, was almost always due to arteriosclerotic changes in the coronaries and had nothing to do with syphilis. Intensive treatment in these cases was dangerous since a too rapid healing of the process about the coronary orifices resulted in scarring and still further narrowing of the openings. A therapeutic paradox was apt to occur. He had never seen syphilis involve the large branches for any extent and thought that specific endarteritis of the terminal branches had been greatly exaggerated.

In about 10 per cent of the cases, aneurysm of the aorta occurred. In many cases of aneurysm he had observed that the heart was not enlarged and that the syphilitic process had not extended to the aortic valve. The valve was, therefore, competent. He thought that the maintenance of a high diastolic pressure, when aortic regurgitation was not present, was an important factor in the production of aneurysm, as in syphilis uncomplicated by arteriosclerosis or nephritis there was only moderate hypertension (150-155 systolic), hardly enough to produce aneurysmal dilatation in a previously weakened wall. The low diastolic pressure in aortic regurgitation often saved the weakened aortic wall from aneurysm. Aside from early supravalvular sclerosis and barring erosion and rupture into adjacent structures, aneurysm was probably the most benign form of cardiac syphilis, since patients could live with large aneurysms for many years.

Provided that the supravalvular sclerosis did not lead to aortic regurgitation, or stenosis of the coronary orifices, or aneurysm, many cases of syphilitic aortitis were benign and were found only at autopsy as historical landmarks, apparently not shortening life.

While he did not deny the existence of various forms of specific myocarditis and was in no position to dispute the careful and painstaking histologic work of Warthin, he was under the impression from both pathologic and clinical experience that the importance of these forms of cardiac syphilis had been greatly exaggerated by some pathologists and by many clinicians. Coronary injury distal to the orifices and a specific myocardial inflammation or pericardial injury in acquired syphilis appeared to him to be of little significance. Aside from rare gummas in the cardiac muscle of appreciable size, he had never seen a specific myocarditis that seriously embarrassed cardiac reserve. Sudden death in cardiac syphilis was almost always due to narrowing of the coronary orifices, to aortic regurgitation or to rupture of an aneurysm.

Book Reviews

RENÉ THÉOPHILE HYACINTHE LAËNNEC: A MEMOIR. By GERALD B. WEBB, M.D., President, Colorado School of Tuberculosis, Colorado Springs; U. S. Government Delegate to the Laënnec Centenary, Paris, December, 1926. Price, $1.50. Pp. 146, with 13 full-page plates. New York: Paul B. Hoeber, Inc., 1928.

Laënnec's short life should be a great inspiration to the medical student and young physician. He began making independent observations when barely 20 years of age and achieved favorable distinction early. He lectured on pathologic anatomy, wrote reviews and abstracts and took part in medical meetings before he obtained his medical degree. He saw that to know disease one must know the underlying changes, and that this knowledge can be gained only by thorough and orderly clinical and anatomic observations. He tells in his own words how he worked: "When a patient enters the hospital it is the duty of a pupil to collect from him those anamnestic facts which he can give concerning his disease, and to follow their course. On examining the patient myself, I dictate the principal symptoms which I observe — those especially which may serve to establish the diagnosis or indications for treatment. And I confirm my conclusion, unless I may have to change it, by subsequent observations. This dictation, which is made in Latin for reasons easily appreciated [Laënnec was an accomplished scholar in the learned languages], is taken down by the pupil in charge of the patient, and at the same time on a separate sheet, which I call the 'diagnostic leaf,' to keep which, in order that it may be shown to me and read whenever required at each visit, is the special duty of another pupil. If a new sign appear, such as might modify the first diagnosis, I have that also added. If a patient die, the account of the autopsy is collected by the pupil in charge of the case. I read this account before all those who have been present at the autopsy, and if any correction is to be made, I make it on the spot, after having consulted with them."

He discovered a new method of observation of universal use in medical practice, and he nearly exhausted the field of investigation by that method, with results of the greatest value. Heretofore, there has not been any easily available complete sketch of Laënnec in English. There is a good sketch of him by John Forbes in his translation, which has appeared in several editions, the fourth in 1834, of Laënnec's "A Treatise on Diseases of Chest and on Mediate Auscultation." In the French there is an excellent biography of Laënnec in two volumes by Alfred Rouxeau which ought to be translated into English. Webb's little book is an elaboration of an address in 1926 to commemorate the centenary of the death of Laënnec, and is reprinted with additions from *Annals of Medical History*, volume 9, 1927. The book traces in a highly interesting way the brief but eventful life of Laënnec (1781-1826) and constitutes the best sketch with illustrations in the English language of one of the greatest of physicians.

LABORATORY DIAGNOSIS AND EXPERIMENTAL METHODS IN TUBERCULOSIS. By HENRY STUART WILLIS, The Johns Hopkins University and Hospital. With a Chapter on Tuberculo-Complement Fixation. By J. Stanley Woolley, Loomis Sanatorium, New York. Introduction by Allen K. Krause, The Johns Hopkins Hospital. Price, $3.50. Pp. 330, with 25 illustrations. Springfield, Ill.: Charles C. Thomas, 1928.

According to the author's introductory remarks, this book "aims to describe the more important methods of the laboratory diagnosis of tuberculosis and to consider some of the more significant procedures and principles involved in the experimental study of the infection." Both aims are well achieved. Any laboratory

worker, whether apprentice or master in the field, will secure reliable results by following carefully the detailed prescriptions in this book, and it may be ascertained as a fact that what Dr. Krause states as a hope in his introduction will "rid a respectable proportion of practitioners of an inferiority complex" in regard to the diagnosis of tuberculosis, by showing them convincingly "that the laboratory diagnosis of the ordinary case of tuberculosis can be accomplished by simple methods in an ordinary physician's hands in an ordinary physician's office." This is accomplished by describing in minute detail every step in the bacteroscopic diagnosis. Other laboratory procedures, such as cultural and inoculation methods and serologic and hematologic technic, are mentioned in full with proper emphasis on their purely auxiliary nature. There is a discussion on the technic, the importance and — last but not least — the limitation of tuberculin tests in diagnosis, which is of high practical value. Experimental methods, such as animal inoculation, including valuable remarks on the anatomy of laboratory animals, and the characteristics of induced tuberculosis, histologic and explanation methods are presented with a clarity which only thorough familiarity with the subject can produce. Theoretical knowledge, the minimum necessary for practical work, is imparted in simple and brief discussions. A second edition, which should follow this book within a short time, should give an opportunity to the author to correct the rather frequent mistakes in spelling of foreign references. The references always include the original articles on each subject, a commendable practice. When it comes to the summing up of the experiences up to the present time, however, the reviewer should like to see, in a few instances, some more modern references than the ones quoted. But in spite of these two points of criticism, it should be emphasized that Dr. Willis' work has set a high standard of practicability and reliability. Dr. Woolley's chapter on tuberculocomplement fixation is well presented, concise and, though cautious in its conclusions, far from pessimistic. A wide distribution of this book would be instrumental in raising the standard of the diagnostic work in tuberculosis.

CLINICAL PHYSIOLOGY (A SYMPTOM ANALYSIS) IN RELATION TO MODERN DIAGNOSIS AND TREATMENT. A TEXT FOR PRACTITIONERS AND SENIOR STUDENTS OF MEDICINE. By ROBERT JOHN STEWART MCDOWALL, D.Sc., M.B., F.R.C.P., Edinburgh, Professor of Physiology, King's College, University of London. With an Introduction by W. P. HALLIBURTON, LL.D., F.R.C.P., F.R.S., Emeritus Professor of Physiology, King's College, University of London. Pp. 383. New York: D. Appleton & Company, 1927.

This book is a readable summary of the views held by the present school of British physiologists in their interpretation of clinical symptoms. It is not a reference book, and clinical syndromes are rarely treated in detail. The bibliography contains only reviews by important workers in the various fields. The parts dealing with the physiology of muscle and nerve, circulation and the respiratory function of the blood are the best, representing the views of Starling, Barcroft, Hill and others. The sections on metabolism, gastro-intestinal physiology, endocrinology and the vitamins are not nearly as good by comparison, and the reader is disappointed not to find reference to many important American contributions in these fields. The book should be of distinct value to the clinician who wishes to acquaint himself with modern conceptions in physiology, but the investigator will find little of service in so short and general a treatise.

Books Received

RENÉ THÉOPHILE HYACINTHE LAËNNEC. A Memoir. By Gerald B. Webb, M.D., President, Colorado School of Tuberculosis, Colorado Springs; U. S. Government Delegate to the Laënnec Centenary, Paris, December, 1926. Price, $1.50. Pp. 146, with 13 illustrations. New York: Paul B. Hoeber, 1928.

FOOD, NUTRITION AND HEALTH. By E. V. McCollum, Ph.D., Sc.D., and Nina Simmonds, Sc.D. (Hygiene), Professor and Associate Professor of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore. Ed. 2, revised. Price, $1.60. Pp. 148. Baltimore: Published by the authors, East End Post Station, box 25.

This book gives a clear, well written and nontechnical account of the newer knowledge of foods and nutrition in relation to health, and it should be given the widest possible distribution.

DIE BIOLOGIE DER PERSON. Ein Handbuch der allgemeinen und speziellen Konstitutionslehre unter Mitarbeit zahlreicher Fachmänner. Von Prof. Dr. T. Brugsch und Prof. Dr. F. H. Lewy. Volume II. Price, 20 marks. Pp. 269, with 18 illustrations. Berlin: Urban and Schwarzenberg, 1928.

INTERNATIONAL CORN BORER INVESTIGATIONS. Scientific Reports. 1927-1928. Edited by Tage Ellinger, Sc.D. Pp. 237. Chicago: International Live Stock Exposition, Union Stock Yards, 1928.

DIE TUBERKULOSE UND IHRE GRENZGEBIETE IN EINZELDARSTELLUNGEN. Beihefte zu den Beiträgen zur Klinik der Tuberkulose und spezifischen Tuberkuloseforschung. Herausgegeben von L. Brauer-Hamburg und H. Ulrici-Sommerfeld. Volume VI. Ausgewählte Schriften zur Tuberkulosepathologie. Von K. E. Ranke Weiland, Professor an der Universität München. Herausgegeben und Eingeleitet von W. und M. Pagel. Price, 20 marks. Pp. 236, with 25 illustrations. Berlin: Julius Springer, 1928.